Asbestos: Modern Insights for Toxicology in the Era of Engineered Nanomaterials

Felley-Bosco, Emanuela ; MacFarlane, Marion

Abstract: Asbestos fibers are naturally occurring silicates that have been extensively used in the past, including house construction, but because of their toxicity, their use has been banned in 63 countries. Despite this, more than one million metric tons of asbestos are still consumed annually in countries where asbestos use has not been banned. Asbestos-related disease incidence is still increasing in several countries, including those countries that banned the use of asbestos more than 30 years ago. We highlight here recent knowledge obtained in experimental models about the mechanisms leading to tumor development following asbestos exposure, including genetic and epigenetic changes. Importantly, the landscape of alterations observed experimentally in tumor samples is consistent with alterations observed in clinical tumor samples; therefore, studies performed on early/precancer stages should help inform secondary prevention, which remains crucial in the absence of an efficient primary prevention. Knowledge gathered on asbestos should also help address future challenges, especially in view of the increased production of new materials that may behave similarly to asbestos fibers.

DOI: https://doi.org/10.1021/acs.chemrestox.8b00146

Originally published at:
Felley-Bosco, Emanuela; MacFarlane, Marion (2018). Asbestos: Modern Insights for Toxicology in the Era of Engineered Nanomaterials. Chemical Research in Toxicology, 31(10):994-1008.
DOI: https://doi.org/10.1021/acs.chemrestox.8b00146

Asbestos: modern insights for toxicology in the era of engineered nanomaterials

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KEYWORDS. Asbestos, asbestos exposure, asbestos-related diseases, mechanisms of asbestos-induced cancer, engineered nanomaterial toxicity.

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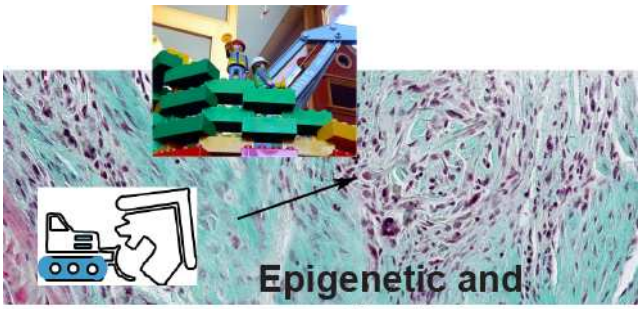

ABSTRACT. Asbestos fibers are naturally occurring silicates that have been extensively used in the past, including in house construction but, because of their toxicity, their use has been banned in sixty-three countries. Despite this, more than one million metric tons of asbestos are still consumed annually in countries where asbestos use has not been banned. Asbestos-related disease incidence is still increasing in several countries, including those countries that banned the use of asbestos more than thirty years ago. We highlight here recent knowledge obtained in experimental models about the mechanisms leading to tumor development following asbestos exposure, including genetic and epigenetic changes. Importantly, the landscape of alterations observed experimentally in tumor samples is consistent with alterations observed in clinical tumor samples; therefore, studies performed on early/pre-cancer stages should help inform secondary prevention, which remains crucial in the absence of an efficient primary prevention. Knowledge gathered on asbestos should also help address future challenges, especially in view of the increased production of new materials that may behave similarly to asbestos fibers.

Introduction

In this perspective our aim is to document that asbestos, although banned in several countries, still represents a threat for human health. After providing information on asbestos exposure, we summarize current knowledge about the mechanisms involved highlighting recent data where similarities with some engineered nanomaterials has been observed. We conclude with open questions that remain to be addressed and suggest future priorities.

Asbestos production and exposure

Asbestos fibers are naturally occurring silicates and their properties include high mechanical and thermal stability, high tensile strength and flexibility, low thermal and electrical conductivity, high absorbency and resistance to acids and bases. The economically relevant ones include amphiboles (crocidolite, amosite, tremolite,anthophyllite and actinolite) and the most commonly used chrysotile, which is a serpentine fiber. Asbestos can be mixed with cement and used in construction and in the past has been widely used in shipbuilding, in household appliances, as a soil conditioner, in cigarette filters, brake linings and theatre curtains.

Annual asbestos production and consumption had peaked in 1980 at approximately 4.8 million metric tons, then decreased to approximately 1.3-1.4 million metric tons by 2016, with Russia being the biggest producer (692,000 tons) followed by Kazakhstan, China and Brazil (contributing for around 200,000 tons), while the biggest consumers are India (308,000 tons), China (288,000 tons), Russia (234,000 tons), followed by Brazil (120,000 tons) and Indonesia (114,000 tons)[1]. In comparison, it is important to note details regarding the use of asbestos in the United States of America (USA), which stopped all production of asbestos in 2002. In 2017,

domestic consumption of imported asbestos minerals was estimated to be 300 tons. It was mostly used in chloralkali industry, which uses asbestos to manufacture semipermeable diaphragms that prevent chlorine generated at the anode of an electrolytic cell from reacting with sodium hydroxide generated at the cathode[1]. In addition, an unknown quantity of asbestos was imported within manufactured products[1].

In 1997, it was estimated that 20% of buildings in the US still contain products made from chrysotile asbestos[2]. Therefore, release of fibers can result from decay, renovation or demolition of these structures. Past industrial production of materials containing asbestos may also lead to contamination of the environment and community exposure (reviewed in [3]). For example, in Libby, Montana, US, vermiculite ore was contaminated with as much as 25% amphibole asbestos and a significant rates of asbestos-related diseases have been observed among community residents who never worked in the vermiculite mining operations.

Based on the recognized association of asbestos exposure with cancer development, which originated from observations done in the 1960's, the use of asbestos is currently banned in 63 countries (http://www.ibasecretariat.org/alpha_ban_list.php).

Based on the pioneering work of Stanton [4], the toxicity of asbestos and asbestos-like fibers is known to be determined by dose, aspect ratio (length/diameter), biopersistence and surface reactivity (reviewed in [5], [6]). The so called classical 'Fiber Pathogenicity Paradigm' is a structure:toxicity model that predicts thinness, length, and biopersistence as key factors that determine fiber pathogenicity [7].

According to the World Health Organization (WHO), respirable fibers have a length above 5 μm, a diameter below 3 μm, and an aspect ratio (length/diameter) above or equal to 3. The occupational recommended exposure limit by the Occupational Safety and Health

Administration (OSHA) is between 0.2 and 1 respirable fiber/cm^3 for an 8 h time weighed average for synthetic mineral fibers (https://www.osha.gov/SLTC/syntheticmineralfibers/table.html) and 0.1 fiber/cm^3 for asbestos (https://www.osha.gov/Publications/osha3095.html).

Lower values exist in other countries (https://www.dguv.de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index-2.jsp). Therefore, assuming an inhalation of ca 6 Mio cm^3/d, and knowing that it has been estimated that 1 µg can correspond, depending on the size of fibers, to up to 10^7 fibers [8], 0.6 µg of respirable fibers are possibly inhaled/day in occupational settings. The fibers then deposit in the nasal, pharyngeal, laryngeal tract or tracheo-bronchial and alveolar tract, depending on whether their aerodynamic diameter is larger or smaller than 0.1 µm (reviewed in [9]). Inhaled fibers are cleared by physiological processes, including phagocytosis by macrophages for fibers shorter than 10-20 µm, and by the lymphatic system. Determination of lung and pleural deposits of asbestos fibers is complex and has only been documented in a few studies (reviewed in [10 11]). Asbestos fibers can adsorb xenobiotics and the deposition of endogenous iron, protein, and mucopolysaccharide on biopersistent fibers results in the formation of ferruginous or asbestos bodies.

For all the reasons highlighted above, it appears that asbestos still represents a worldwide threat and awareness about its toxicity (as discussed below) needs to be of public concern.

Asbestos-related diseases

Exposure to asbestos is linked to non-malignant diseases and cancer. Wagner[12] pioneered epidemiological studies investigating asbestos-related malignant mesothelioma, but the relationship between asbestos exposure and asbestosis and lung cancer has been known since the early past century[13, 14] (reviewed in [15]). Non-malignant diseases include pleural plaques, a form of

localized area of pleural thickening, pleural fibrosis, benign effusion and asbestosis [16]. Asbestosis, is characterized by lung inflammation and scarring and life expectancy averages 3 to 5 years after diagnosis. Asbestosis has been shown to cause death in 10% of insulation workers [17]. Although as a result of regulated control of asbestos exposure in the workplace the prevalence of asbestosis has decreased, the estimate of the global number of asbestosis deaths from the Global Burden of Disease (determined in 2016) is 3495[18]. Worldwide asbestosis death rate is increasing including in high-income countries (Figure 1).

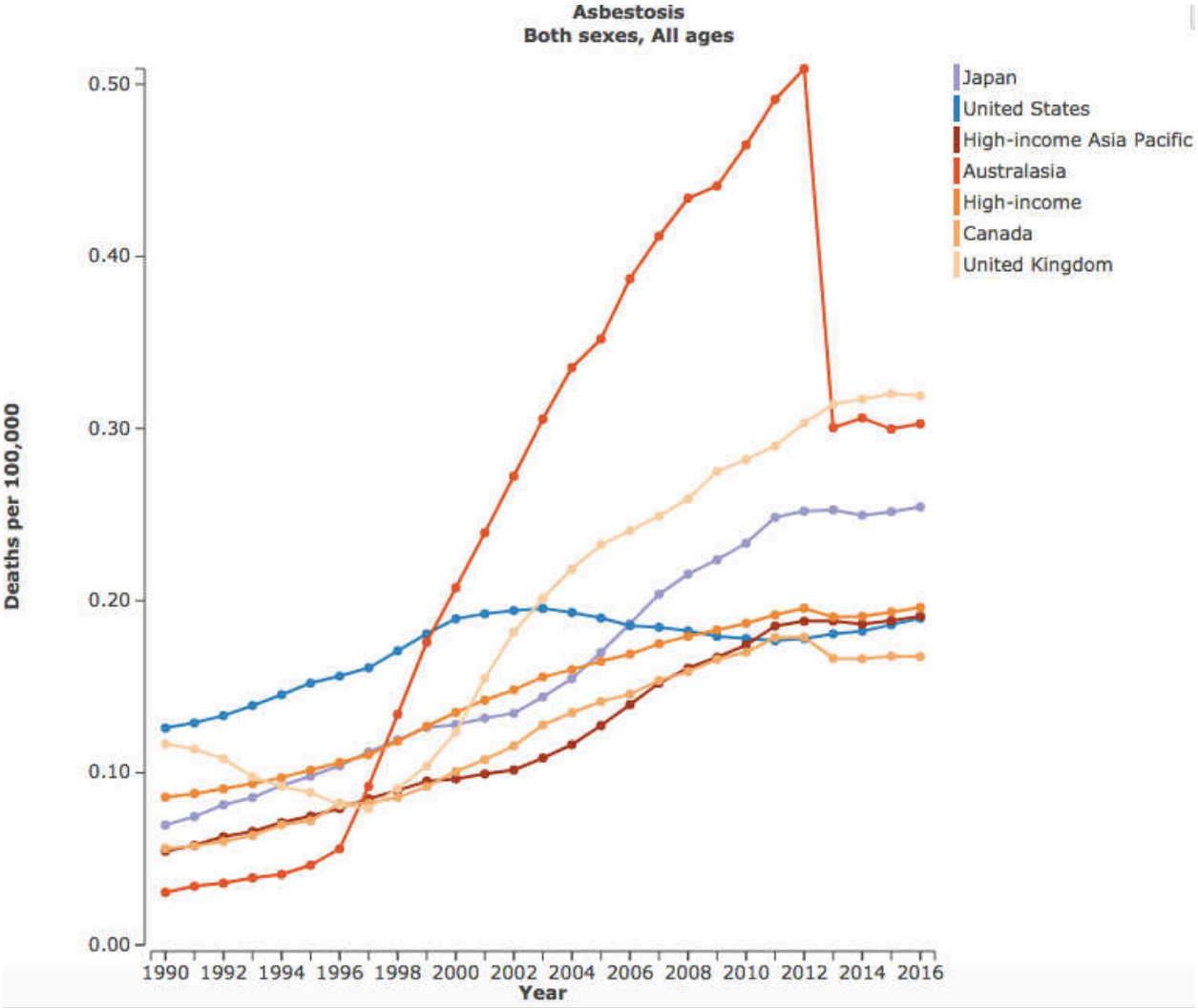

Figure 1. Asbestosis mortality rates are still increasing worldwide including in high-income countries (https://vizhub.healthdata.org/gbd-compare/ , accessed July 31 2018) [19].

The association of pleural plaques with pleural mesothelioma is still not clear. Pleural plaques have been considered as "sentinel events" for asbestos exposure, although the presence of pleural plaques has no correlation with the development of mesothelioma [20]. However, a study has shown a statistically significant association between mesothelioma and pleural plaques [21].

Malignant diseases related to asbestos exposure (reviewed in [17]) include lung cancer, mesothelioma, ovarian cancer, and laryngeal cancer. More recently, data also support the association between occupational exposure to asbestos and stomach and colorectal carcinomas[22, 23]. All these cancers are found in the scientific literature to be in excess among asbestos-exposed individuals.

Since the seminal experiments of Wagner[24], exposure to asbestos has been clearly identified as cause of mesothelioma. Malignant mesothelioma is a rapidly fatal and highly resilient tumor arising in the thin layer of tissue known as the mesothelium, which has mesodermal origins and covers many of the important internal organs like the lungs (pleural mesothelioma), peritoneal cavities (peritoneal mesothelioma), the sacs surrounding the heart (pericardial mesothelioma) and the testis (tunica vaginalis mesothelioma). Malignant pleural mesothelioma (MPM) is the most common type accounting for about 80% of cases, because most exposures result from inhalation. However, an excess of peritoneal mesotheliomas is also found in asbestos-exposed workers. Although MPM is a relatively rare cancer in the general population, individuals occupationally exposed to asbestos, are at a higher risk for contracting this disease[25-31]. It is estimated that 1.3 million workers in the USA and 125 million people worldwide have a history

of asbestos exposure[32]. Considering the rising use of asbestos in developing countries, it is likely that this number will continue to increase in the future[3, 17, 33]. Familial, genetic and environmental factors may also contribute to the incidence of MPM[34-37].

The incidence of MPM in 2011-2012 was around 3/100,000 in men in Belgium, Switzerland and Denmark and 4/100,000 in the Netherlands, while it is between 1 and 2/100,000 in Austria and Sweden. The incidence in women, while similar in all those countries, is much lower (ca 0.5/100,000) (Figure 2).

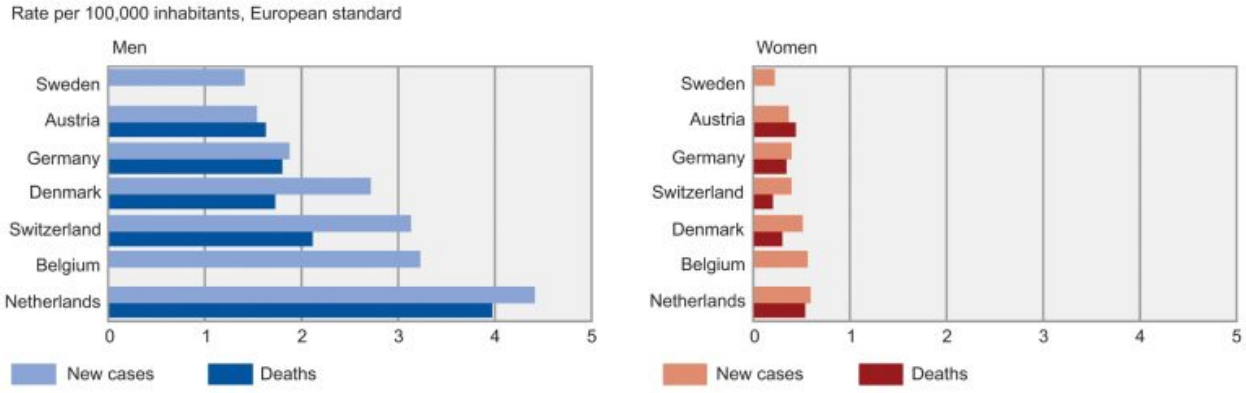

Figure 2. Mesothelioma international comparison 2011-2012. Corresponds to the ICD-10 Code C45 with the exception of Sweden and Denmark (C38.4). Data from FSO/NICER 2008-2012 were used for Switzerland (C38.4, C45.0). Belgium and Sweden: no comparable data on mortality. Norway, Italy and France: no data available. (https://www.bfs.admin.ch/bfs/de/home/statistiken/kataloge-datenbanken/publikationen.assetdetail.350143.html) [38].

The rate reported for the UK is higher with a similar difference between men and women (Figure 3).

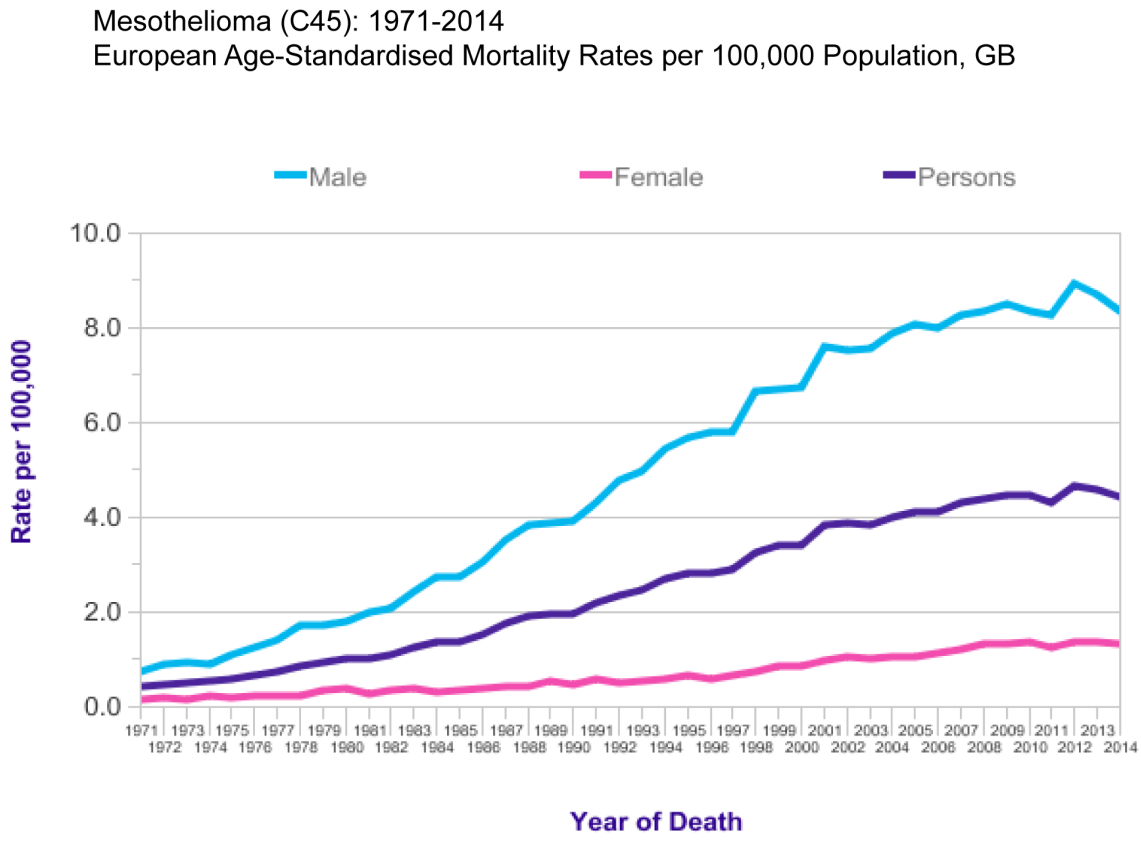

Figure 3. Mesothelioma mortality rates have increased by 887% in Great Britain since the early 1970s. Cancer Research UK; http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/mesothelioma/mortality#heading-Two

Worldwide mesothelioma death rate is increasing including in high-income countries (Figure 4).

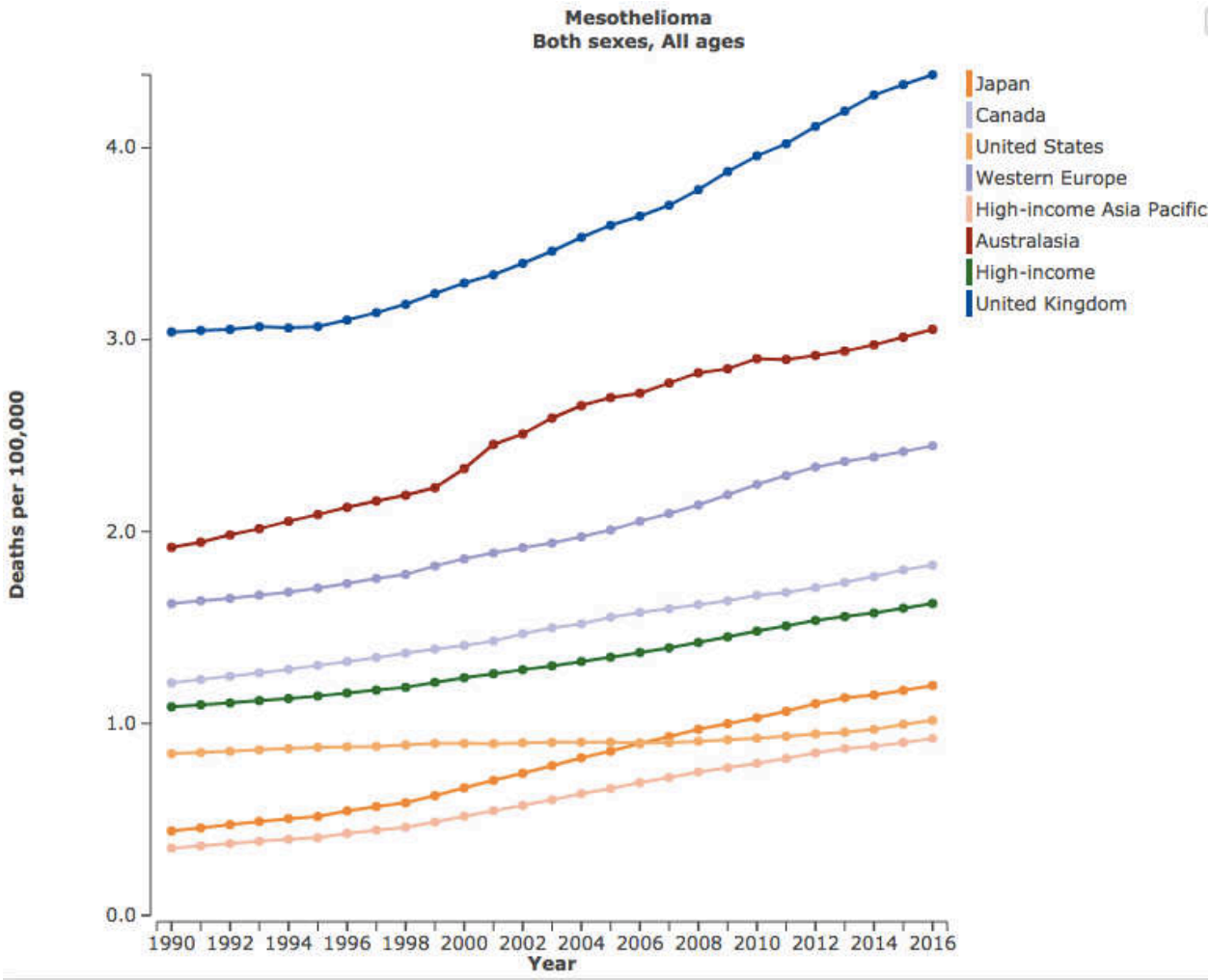

Figure 4. Mesothelioma mortality rates are still increasing worldwide including in high-income countries (https://vizhub.healthdata.org/gbd-compare/ , accessed July 31 2018) [19].

The annual global health care cost associated with asbestos-related cancer has been estimated to amount to 2.4-3.9 billion USD worldwide [39], which, although 100-fold lower compared to smoking attributable illnesses [40], will not decrease soon taking into account those countries that still produce/ consume asbestos. In addition, even in countries that never produced asbestos, such as Iceland [41], and others where asbestos was banned early, the incidence of MPM is still rising. In Sweden, where asbestos was banned in 1982, no clear effect on the occurrence of mesothelioma has been found in a Swedish Nordic Occupational Cancer Study[42]. An additional important factor

is that the incidence of mesothelioma is higher and plateauing in a population (Figure 5) age range (70-85+), that is rapidly increasing worldwide (http://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2017_Highlights.pdf).

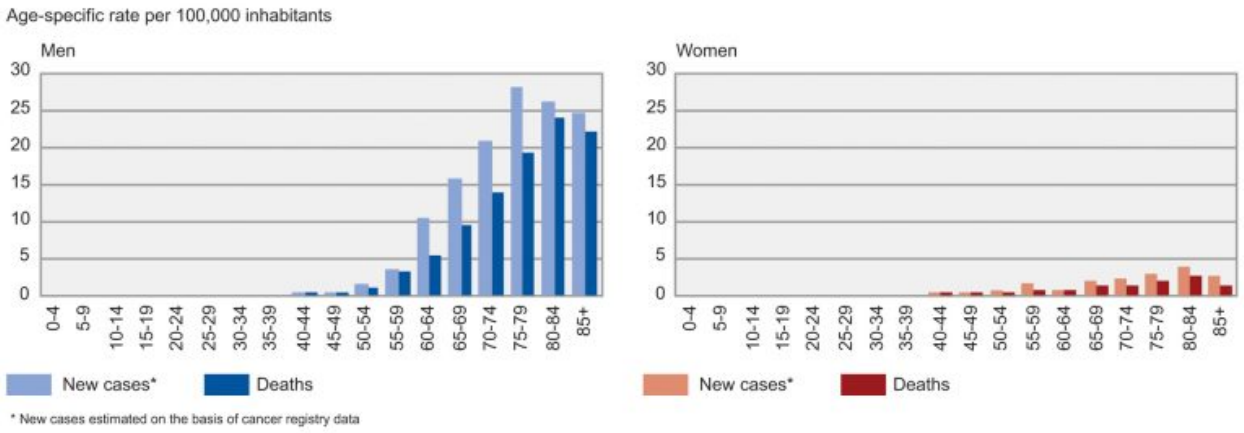

Figure 5. Pleural mesothelioma by age, in Switzerland 2008-2012. * New cases estimated on the basis of cancer registry data. (https://www.bfs.admin.ch/bfs/de/home/statistiken/kataloge-datenbanken/publikationen.assetdetail.350143.html) [38].

The increase in mesothelioma incidence rate in older populations has been used as additional support for the long latency period between known asbestos exposure and cancer development [43] that may contribute to the observed continued increase in mesothelioma incidence. Of the worldwide mesothelioma deaths reported to the WHO between 1998 and 2008, 54% occurred in Europe [44] highlighting the problem of underreporting in some countries, as it is often observed with rare diseases that are difficult to diagnose. In addition, a specific International Classification

of Diseases (ICD) code for mesothelioma, which is the foundation for global statistics, has been available only since the tenth revision (ICD10), which was first implemented in 1994. Many countries have not yet implemented ICD10, and the accuracy of coding varies by country.

Mechanisms of MPM development

The mechanism of development of MPM after exposure to asbestos fibers is not fully elucidated but several hypotheses can be suggested based on experimental data and observations in clinical samples[45]. The aim of this perspective is first to briefly summarize these data, some of which has been extensively detailed in several reviews [7, 46-52].

Inhaled asbestos fibers pass the alveolar barrier and reach the lung interstitium. During this process they transiently activate signaling pathways such as the NOD-like receptor protein 3 (NLRP3) inflammasome [53] , or other yet uncharacterized pathways, in alveolar and/or interstitial macrophages and the transcription factor nuclear Factor Kappa B subunit 1 (NF-κB) in lung epithelial cells, resulting in the release of pro-inflammatory cytokines. The mechanism underlying the passage of fibers from the lung interstitium to the pleural space is matter of controversy [7, 48]; however, what seems generally accepted is that fibers accumulate at the site of pleural fluid drainage. The latter takes place through the lymphatic stomata which are ovoid or round openings of 2 to 10 μm in diameter found on small selected surface areas of the parietal pleura, particularly the anterior lower chest wall and mediastinum directed part [54]. No stomata are present on the visceral pleura. The accumulation of fibers at parietal pleura supports the concept that mesothelioma originates primarily from the this site [55]. The persistent accumulation of fibers into the pleural space results in chronic damage and inflammation of the mesothelial surface lining cells, or the immediate submesothelial layer, respectively. Although it is not

known which cells are at the origin of this cancer there has been one study which supports the concept of multiclonality [56].

Genotoxic initiation processes (reviewed in [8, 57]) and/or an epigenetic mechanism mediate the carcinogenic activity of asbestos. Asbestos fibers phagocytosed by dividing cells interfere with mitosis [20]. Asbestos fibers induce an increase in DNA breaks *in vitro* resulting in ~50% increase in loss of heterozygosity in exposed lymphocytes [58] or micronuclei formation in exposed lung epithelial cells [59]. The capacity of asbestos to generate DNA breaks is strongly linked to the presence of fiber-associated iron and reactive oxygen species (ROS) generation [60]. *In vivo,* asbestos induced two to three-fold increased levels of 8-hydroxydeoxyguanosine compared to basal tissue levels[61, 62]. A 50% increase of G to T transversion[63], or mere doubling of spontaneous mutations appearing 16 weeks after exposure [64], have been described in *lacI* transgenic rats depending on exposure route, indicating that most probably also *in vivo* DNA breaks are the main genotoxic consequence of iron-loaded asbestos fibers. The consequence, at least *in vitro*, of this overall low but persistent DNA damage in non-neoplastic cells is senescence [65], characterized by stable cell cycle arrest with active metabolism. Senescence is a delayed stress response involving multiple effector mechanisms including DNA damage response [66, 67] epigenetic regulation [68] , autophagy [69] and senescence-associated secretion phenotype [70-72, 73] . It has been proposed that the senescence-associated secretory phenotype, might stimulate the immune system to clear senescent cells (reviewed in [74]). One reason why senescent cells have to be eliminated is that they can secrete mitogenic factors [71]. Senescent phenotype, due to low level of persistent DNA damage in mesothelial cells which are not efficiently cleared by the immune system, may lead to epigenetic effects. This is supported by

the recent observation that carcinogenic asbestos fibers induce methylation of the *cyclin-dependent kinase inhibitor 2A* (CDKN2A) promoter [62] in the early stages of tumor development.

In addition, Rehrauer et al[75] observed, in samples from experimental animals, that asbestos increased the levels of RNA mutations and the most abundant changes were A to G mutations, likely resulting from hydrolytic deamination of adenosine downstream of adenosine-deaminase acting on RNA (Adar) activity [76] (I is detected as G in RNA-sequencing). Interestingly, Adar is a target of the interferon type 1 pathway, which acts as a negative feedback regulator to avoid autoimmunity, an effect which has recently been linked to asbestos amphiboles (reviewed in [77]) and further highlighting the immunotoxicity of asbestos.

The reviews mentioned above and [78] summarize all the experimental evidence of tissue repair stimulation following exposure to asbestos fibers or mesothelium injury. In a recent study comparing pre-cancer and cancer stage in mice following exposure to crocidolite fibers, it was documented that chronic tissue repair activates stem cell signaling pathways to regenerate the tissues [75]. Because of persistent stimulation, oncogenic events have been postulated to occur in such conditions, leading to the development of a tumor [79]. Stem cells may be involved in this (these) process(es). Two scenarios can be imagined. On one hand, it is possible that a differentiated mesothelial cell starts dividing and dedifferentiates re-activating stem cell signaling present during embryonic development. On the other hand, mesothelial precursors might be present. The only embryonic mesothelial precursor population described to date is a $Mesothelin^{+}CD90^{+}CD34^{+}$ population [80] and an increase in a cell population with similar characteristics has been described in the peritoneal lavage collected after intraperitoneal administration of crocidolite fibers [75]. However, lineage-tracing studies would be necessary to learn whether these cells are at the origin of mesothelioma.

In order to understand which pathways are important for mesothelioma development, an approach complementary to experimental exposure is the analysis of clinical samples. A recent large-scale study, based on pathological samples, has comprehensively characterized the most frequent genetic alterations in MPM [81], which involve tumor suppressor inactivation, mediated by multiple mechanisms that include single nucleotide variants, copy number losses, gene fusions and splicing alterations. According to the catalogue of somatic mutations in cancer (COSMIC, cancer.sanger.ac.uk version 85), the four most commonly mutated genes in MPM are: the tumor suppressor *CDKN2A*, followed by *BAP1* (*BRCA1-associated protein 1*), *NF2* (*neurofibromatosis type 2*) and *TP53,* and mice deficient for one of these genes display an increased incidence of mesothelioma after exposure to asbestos fibers [82-86]. Pathways most altered include histone methylation, as well as the Hippo signaling pathways (see below). Compared to other cancer MPM display a relatively low number of point mutations in cancer genes, however there is a global tumor suppressor inactivation, which is associated with promoter CpG methylation, asbestos burden and worst clinical outcome [87].

CDKN2A/B

Genetic alterations in the chromosomal region including *CDKN2A* (*INK4a/ARF)* and *CDKN2B* have been observed in 82% and 76% respectively of human and 56% and 60% respectively of experimental mouse mesotheliomas [88] . Human mesotheliomas lack the expression of the *INK4a/ARF* locus-encoded $P16^{INK4A}$ and $P14^{ARF}$ proteins [89], [90] due to gene deletion [91], [92], [93] or methylation [94], [95], [96]. Moreover, *CDKN2A* loss has been shown to be associated with shorter patient survival[97-99], and with non-epithelioid histology[100].

BAP1

Somatic *BAP1* mutations in MPM were first described by Bott et al. and at about the same time Testa et al. reported on germline *BAP1* mutations predisposing to several cancers including malignant mesothelioma and renal cancers, which are derived from the mesodermal lineage, but also uveal melanoma [34, 101]. Nowadays the disorder is referred to as BAP1 tumor predisposition syndrome in the Online Mendelian Inheritance in Man (https://www.omim.org/) database [102]. The prevalence of germline mutations in sporadic malignant pleural mesothelioma patients is around 1-2% [103-106]. Therefore, germline *BAP1* mutation seem to have a minor role in the pathogenesis of sporadic malignant pleural mesothelioma.

BAP1 protein has multiple functions and somatic mutations might play a role in the neoplastic process. Contrary to *CDKN2A*, *BAP1* mutations appear be associated with improved patient survival [107,108]. BAP1 is part of deubiquitinating enzymes, which remove ubiquitin from different targets, thereby opposing the function of E3 ubiquitin ligases [109];[101];[110]. BAP1-deficient cells are sensitive to ionizing radiation (IR) and poly(ADP-ribose) polymerase inhibition [111];[112]. Additionally, BAP1 is recruited to double-strand break sites and it is suggested to regulate proteins involved in homologous recombination, such as Breast Cancer 1 (BRCA1) and RAD51 recombinase [112];[113]. Moreover, proteomic analyses revealed that BAP1 is phosphorylated upon DNA damage on ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) kinases consensus sites [114, 115]. Phosphorylation on multiple sites seems to be advantageous for cell survival after IR [112]. BAP1 dimer was found to form two different complexes with the transcriptional regulators ASXL1 and ASXL2, human homologs of Drosophila additional sex combs, which are both able to deubiquitinate histone monoubiquitinated at K119 (H2Aub) [116];[117];[118]. H2Aub is involved in transcriptional regulation, frequently correlated with gene silencing and it additionally occurs at sites of DNA damage and

is important for X chromosome inactivation [119, 120]. However, BAP1 activity may result in gene silencing since by forming a complex with Forkhead Box K2 (FOXK2) transcription factor it leads to repression of some FOXK2 target genes [121]. BAP1 was also found to be involved in metabolism by the stabilization of peroxisome proliferator activator receptor-γ coactivator 1α protein, a master regulator of mitochondrial biogenesis and promoter of oxidative metabolism [122];[123]. In summary, BAP1 controls several signaling pathways and its function might context dependent.

NF2

The *NF2* gene was discovered by mapping hereditary disease Neurofibromatosis type 2 that predisposes to acustic neuromas, neurofibromas and meningiomas. Mutations in the *NF2*-encoded tumor suppressor Merlin, have been found in 40% of human MPM [124],[125],[126, 127]. Mesotheliomas develop more frequently after inactivation of one *Nf2* allele, as compared to wild-type animals in mice experimentally exposed to asbestos fibers [82, 83]. Moreover, the remaining *Nf2* allele is lost, indicating that the Nf2 function alterations have a "driver" role in asbestos-induced mesothelioma [82] when accompanied by a loss of *Ink4a/Arf* [128] and *Cdkn2b* [83]. The essential role of loss of cell cycle control and NF2 function in mesothelioma development has been confirmed in experimental animal models where *Nf2* was inactivated by adeno-Cre infection of the mesothelial cells lining the thoracic cavity in a "permissive" (*Ink4a/Arf*-deficient) and/or *p53* deficient background [129].

The reason why *NF2* is an ideal oncogenic target during the development of MPM is that NF2 translocates into the nucleus, where it inhibits the cullin-RING E3 ubiquitin ligase 4 (CRL4) and as a result controls a subset of Hippo pathway target genes [130]. Recent data [81] has confirmed that NF2/Hippo signaling is disrupted in most cases of MPM [127, 131] [101]. Key components of the Hippo

pathway include two kinases: Mammalian Sterile20-like and Large Tumor Suppressor. The sequential activation of these kinases leads to phosphorylation of the transcription factor YAP. When Hippo signaling is reduced, e.g. when NF2 signaling is disrupted, YAP phosphorylation decreases, leading to its nuclear localization and regulation of target genes. Rehrauer et al recently described progressive YAP activation during mesothelioma development in an experimental animal model [75] and low nuclear merlin expression in patient tumor tissues obtained at surgery after cisplatin/pemetrexed chemotherapy is associated with worst overall survival[132].

TP53

One interesting feature of tumors bearing *TP53* mutations is that the genome profile is 75% haploid and this profile seems to occur in younger and female patients (communicated by Dr. Ladanyi at International Mesothelioma Interest Group (iMig) Meeting 2018). This suggests that mitosis of a haploid cell has been permitted by the loss of TP53 function, which would otherwise be activated by errors during the segregation of the chromosomes and lead to cell death [133]. Interestingly these patients have also mutations in the histone methyltransferase SETDB1 consistent with what was previously observed in one patient with MPM and two additional primary cancers [134].

Considering that the two genes discussed above, *BAP1* and *NF2*, are both associated with genetic diseases, one may ask the question why e.g. mesothelioma is not a feature of hereditary NF2 patients or why patients with BAP1 tumor predisposition syndrome develop tumors of non-mesodermal derived tissue. As hypothesized by Knudson 23 years ago[135], pathological conditions like the one induced by exposure to asbestos are necessary for stem cell proliferation by some

epigenetic means followed by spontaneously occurring mutations. This would be consistent with the observed genetic signature of mutations observed in mesothelioma where 50% of mutation signatures correspond to no predominant transitions or transversions and 25% correspond to CpG deamination [81], which are spontaneously occurring mutations. Pathological conditions induced by exposure to asbestos include events leading to immunosuppression favoring the growth of mutated cells[75] indicating a possible way forward so-called "secondary prevention", i.e. early tumor detection, but for the time being biomarkers for early detection are still missing[136]. However, the recent observation[137] of single-layered surface mesothelial proliferations with deletion of *CDKN2A* and *BAP1,* likely representing mesothelioma *in situ,* should encourage this approach.

Altogether, experimental investigation of asbestos-induced effects and analysis of clinical samples have been useful to get more insights into the development of cancer following asbestos exposure (Figure 6); however we have highlighted several issues that still need to be addressed and which may also be useful to assess the toxicity of new materials, with asbestos still present, as discussed in the next section.

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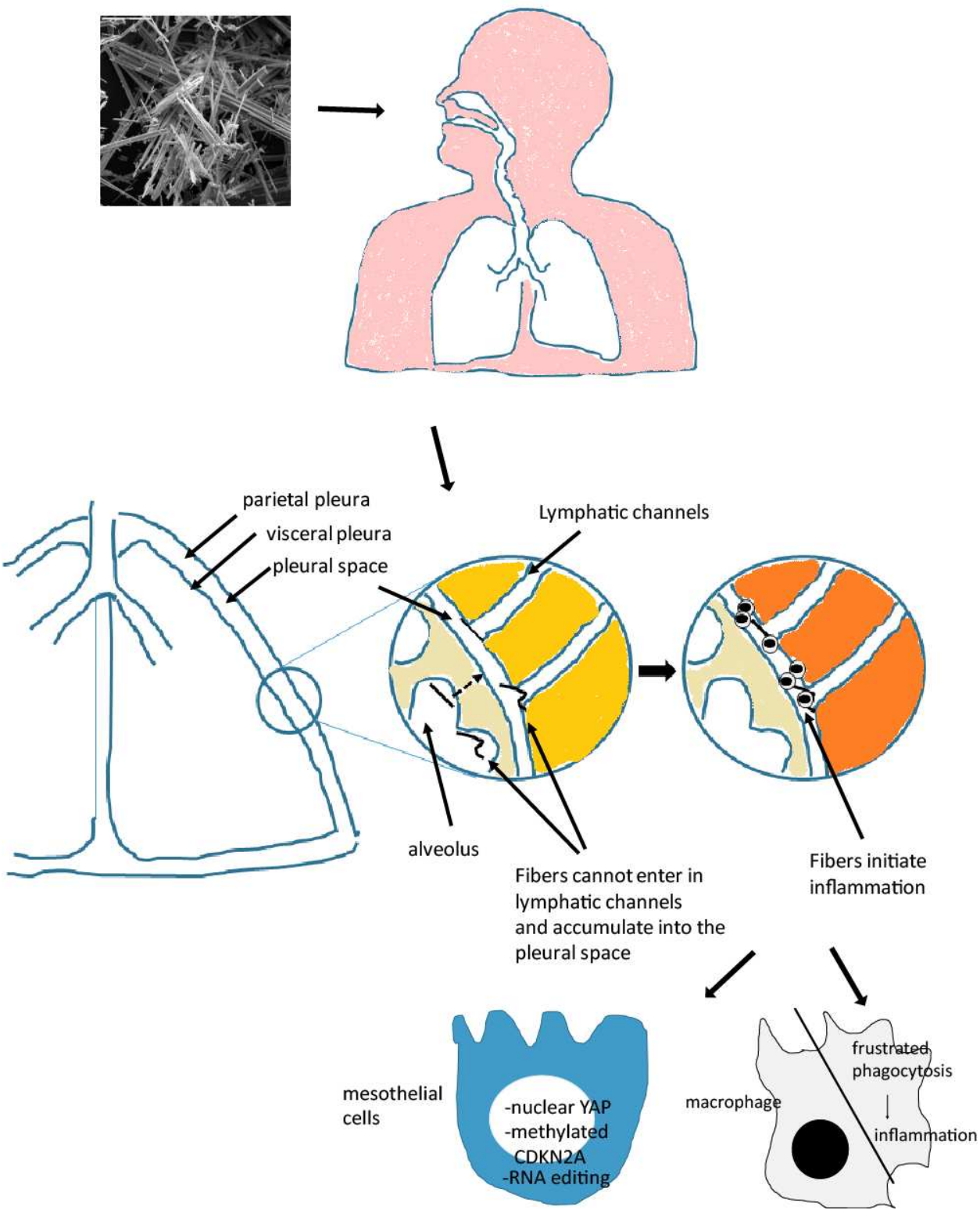
parietal pleura
visceral pleura
pleural space
Lymphatic channels
alveolus
Fibers cannot enter in lymphatic channels and accumulate into the pleural space
Fibers initiate inflammation
mesothelial cells
-nuclear YAP
-methylated CDKN2A
-RNA editing
macrophage
frustrated phagocytosis
inflammation

Figure 6. Hypothesized mechanism of mesothelioma development. After inhalation, carcinogenic fibers arrive in the pleural space, after passing the alveolar barrier and the visceral pleura. Due to their physical properties, they can not negotiate the stomata openings of lymphatic vessels of the parietal pleura and are retained in the pleural space, where they initiate inflammation linked to frustrated phagocytosis and likely other mechanisms, leading to increased nuclear YAP, methylation of CDKN2A promoter and RNA editing (adapted from [7]).

Engineered Nanomaterials

An aspect worth considering is that the knowledge that has been acquired to date on asbestos fibers could help prepare for future challenges – in particular, with the increased production of materials that behave similarly to asbestos fibers. In this regard, engineered nanomaterials present remarkable opportunities for industrial growth and development through their applications in medicine, electronics and numerous other areas[138]. However, there are considerable gaps in our knowledge concerning the hazardous effects of engineered nanomaterials on both human health and the environment.

Carbon nanotubes (CNT) are being used in an increasing number of fields ranging from rechargeable batteries to high performance structural materials and confirmed production and use of carbon nanotubes was evaluated to be of ~2000 tons in 2011 [139] but is rapidly increasing. Multi-walled carbon nanotubes (MWCNTs) are made of concentric layers of graphene sheets. Their cylindrical nature, exceptional mechanical strength and intrinsic physico-chemical properties, render their feasibility for use in a number of applications [140]. Carbon nanotubes can exist as compact bundles if they 'grow' as tangles (like balls of string), and whilst CNT in such bundles are tubular as far as cells are concerned they are particulate. However, if CNT are pristine the tubules can grow straight and the CNT can adopt a fibrous, 'high-aspect' shape. Not

surprisingly, this has raised concerns about their potential adverse health effects; indeed, in 2014, IARC classified MWCNT-7 (Mitsui Ltd, Japan) to category 2B: as possibly carcinogenic to humans. While the National Institute for Occupational Safety and Health recommended exposure limit for CNT is 1 $\mu g/m^3$ for the respirable size fraction (8h time weighted average)[141], the main question in the modern era of nanotoxicology is whether nanomaterials of different types with a fibrous shape, conform to the classical 'Fiber Pathogenicity Paradigm', knowing that some induce lung cancer and mesothelioma in animal experiments. Evidence demonstrating that length is a key factor in the pathogenicity of nanomaterials comes from a number of sources, including toxicological studies in rodents where it is possible to characterize the length of the fibers or define length categories and assess their effects [142-146]. Additional parameters affecting the pathogenicity of nanofibers include diameter[147] and mechanical bending stiffness[141].

The role of clearance in fiber effects is well understood and has been shown to have a profound effect on the biopersistence and toxicity of fibers. Studies exploring the durability of single-walled and MWCNT samples in simulated phagolysosomal fluid have shown that a loss of mass and fiber shortening is paralled by a loss of pathogenicity [148]. Importantly, biosolubility in enhanced by surface modification of the carbon nanotubes thereby offering the opportunity to reduce the potential toxicity [149].

The accumulation of long fibers in lung tissue can lead to numerous adverse responses which if sustained may result in lung pathologies as discussed for asbestos (see above). Apart from recent studies conducted in a large-scale MWCNT manufacturing facility in Russia, which revealed the accumulation of inflammatory and fibrotic biomarkers in biofluids of workers manufacturing MWCNTs [138, 150], lung disease directly associated with CNT exposure has not been widely investigated in human populations. On the other hand, several *in vivo* rodent studies

examining the lung toxicity of CNT have revealed a pattern of effects similar to those observed following exposure to asbestos [151, 152 153, 154]. Although of the majority of these studies were investigating short-term effects, short-term endpoints such as genotoxicity, inflammogenicity and fibrogenicity may still have the capacity to predict long-term carcinogenicity.

To date, the presence of lung tumors in CNT-exposed animals has only been demonstrated following inhalation exposure to MWCNT-7 in mice pretreated with the chemical initiator 3-methylcholanthrene [155, 156]. Importantly, these effects were observed at exposures of 31 μg/mouse – a dose that is achievable in terms of human exposure in occupational settings. More recently, Suzui et al[157] have also reported lung tumors and mesothelioma in rats exposed to MWCNT-N by trans-tracheal intrapulmonary spraying. However, it is unclear whether the data obtained using MWCNT-7 can be extended to other CNT due to their heterogeneity[6, 141].

In a number of studies, the pro-inflammatory response to CNT in the lung has been attributed to ROS generated by the presence of contaminating transition metals, or structural defects in the CNT generating free bonds [158].

Alternatively CNT can increase lung ROS and oxidative stress by activating inflammatory cells recruited to the site of fiber deposition and frustrated phagocytosis (as discussed above for asbestos fibers). The latter is accompanied by the release of oxidants and cytokines, as well as lysosomal destabilisation[159], increasing recruitment of inflammatory cells to the lungs and activating the surrounding epithelial cells, leading to an inflammatory response. The ability of long CNT to stimulate frustrated phagocytosis in macrophages *in vitro* has been described in studies, which have shown specific length-dependent increases in the release of superoxide anion and pro-inflammatory cytokines [143].

Fibers may also be directly genotoxic by physically interfering with mitosis. Indeed, various genetic abnormalities including multiple mitotic spindle poles, anaphase bridges and aneuploidy have been observed after *in vitro* exposure of airway epithelial cells to single-wall CNT [160, 161].

As described above, in addition to mesothelioma, other forms of pleural diseases, such as pleural effusion and pleural plaques, are recognised as asbestos exposure-related disease in the pleural space. The length-dependent relationship between CNT exposure in the pleural space and disease development has been explored using a model of direct pleural injection. Murphy et al[144] demonstrated that CNT, like asbestos, exhibit length-dependent pathogenicity. A follow-up study, using purpose-synthesized silver/nickel nanowires with distinct length classes, revealed a cut-off length ≥5 μm for nanowires being inflammogenic[145]. Even though these *in vivo* studies on predicting the pathogenicity of high-aspect ratio nanomaterials in relation to asbestos fiber exposure in the pleural space were short term studies, they highlighted that if fiber-containing CNT are long enough and biopersistent they may pose an asbestos-like hazard. Recently, this question was directly addressed by Chernova et al [62], who compared the carcinogenicity of long and short MWCNT up to 18 months after direct intrapleural injection. This study demonstrated that long-fiber CNT, like asbestos, exhibit length-dependent pathogenicity and pleural disease including mesothelioma and pleural fibrosis. These findings are consistent with previous *in vivo* mouse studies, where peritoneal exposure to CNT was used as a surrogate for the pleural cavity. Thus, in genetically-accelerated or peritoneal-exposed rodent models, CNT induced length-dependent inflammation, accumulation of macrophages leading to the formation of granulomas[162], and in long-term studies mesothelioma[147, 163].

While most *in vivo* studies that have assessed the carcinogenic hazard of CNT in rodents have been limited to obtaining histological evidence of tumor development, Chernova et al[62] examined

for the first time whether the molecular mechanisms underlying long-fiber CNT-induced pleural carcinogenesis faithfully replicate human mesothelioma. Importantly, the results showed that long-fiber CNT cause sustained inflammation and progressive fibrosis of the parietal pleura, which in the case of both inflammatory lesions and CNT-induced tumors is accompanied by disruption (via epigenetic mechanisms) of the key tumor suppressor gene, Cdkn2a. Moreover, this finding was consistent with Nagai et al[147] who had reported homozygous deletion of Cdkn2a/Cdkn2b in rats peritoneally exposed to MWCNTs. Notably, although co-deletion is not always specified as both genes are located on the same locus, this co-deletion is often found in human mesothelioma, as well as in murine asbestos-induced mesotheliomas. Together, these findings highlight a potential role for epigenetic regulation in connecting fiber (including CNT)-induced chronic inflammation with mesothelioma development.

In summary, due to their useful physical and chemical properties, nanomaterial manufacture is an area of industrial growth. In the case of CNT, these can be manufactured as tight tangles of nanotubes that are essentially particles, or as high-aspect ratio 'fibers'. It is therefore important to note that the effects of CNT as particles would likely be limited to the lungs (fibrosis and cancer), whereas CNT as fibers would have pulmonary effects but also affect the pleura (fibrosis and mesothelioma) – crucially, these effects would be predicted to be length-dependent. It should however be noted that fiber/particle biodistribution requires further study as they might also reach other sites, even after inhalation (for example, as in the case of digestive cancers linked to asbestos exposure). In support of this, recent in vivo findings suggest long-fiber CNTs that are biopersistent pose an asbestos-like hazard. Therefore, the potential for human exposure and subsequent disease development is of major concern.

Open questions and future priorities

Open questions concerning asbestos include the lack of information and reporting about exposure and related toxicity in some parts of the world, including community exposures resulting from the contamination of the environment. In some developed countries the absence of adequate measures to enforce primary prevention e.g. during renovation or demolition of structures that can release asbestos fibers was also highlighted at the iMig meeting 2018.

Studying the toxicity of fibers is complicated by the fact that for a given mineral, at a given amount of material, the number of fibers will depend on their size, and the latter will also influence their toxicity. This has to be kept in mind for a correct interpretation of published studies, in addition to other factors such as surface's adsorbed contaminants and should be taken into account for future studies.

Diseases arising from exposure to asbestos or asbestos-like materials are likely to depend on which cells are damaged and therefore knowledge about the sites of fiber biodistribution/biopersistence is essential.

In experimental models, the observation of epigenetic silencing of *CDKN2A* as an early event after exposure to asbestos fibers raises the question of the activity of chromatin modifiers and possibly a role for long-non-coding-RNA in asbestos carcinogenicity.

Even if as discussed above a lot of knowledge has been acquired on the function of the NF2/Hippo and BAP1 pathways, it is still necessary to understand why and how these two pathways in particular are disrupted after exposure to carcinogenic fibers.

The reason why some individuals develop malignant disease at an earlier age includes loss of function of TP53, which allows haploid cell survival, and this is a novel mechanistic aspect to add to the known ability of asbestos to interfere with the mitotic spindle, mostly linked to

polyploidy. This novel aspect requires further investigation. In this context it is worth noting that, in *apoptosis associated speck-like protein containing a CARD* (*Asc)* hemizygotes mice, a delay in mesothelioma appearance after exposure to asbestos was observed compared to wild-type mice. Because Asc forms a complex with absent in melanoma 2 (AIM2), which senses DNA breaks[164], it may be worthwhile to investigate whether AIM2 deficiency has an influence on mesothelioma development.

Future priorities include secondary prevention of malignant disease i.e. early tumor detection that is completely missing for the time being.

Altogether asbestos and new materials with similar fiber-like behavior still represent a major threat to human health and we suggest that mechanistic studies using standardized benchmark asbestos alongside newer materials of potential concern are still necessary.

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Author Contributions

The manuscript was written through contributions from both authors. Both authors have given approval to the final version of the manuscript.

Funding Sources

The laboratory of EFB is supported by the Stiftung für Angewandte Krebsforschung, the Walter-Bruckerhoff Stiftung and the Swiss National Science Foundation (CRSII3_147697). The laboratory of MMF is supported by the UK Medical Research Council (MRC).

ABBREVIATIONS

Adar, adenosine-deaminase acting on RNA; AIM2, absent in melanoma 2; Asc, apoptosis associated speck-like protein containing a CARD; ASXL1 and ASXL2, human homologs of Drosophila additional sex combs 1 and 2 ; ATM kinase, ataxia telangiectasia mutated kinase; ATR kinase, ataxia telangiectasia and Rad3 related; BAP1, BRCA1-associated protein 1; BRCA1, breast cancer 1; CD34, cluster of differentiation 34 ; CD90, cluster of differentiation 90; CI, Confidence Interval; CNT , Carbon nanotubes ; CRL4, cullin-RING E3 ubiquitin ligase 4; ICD, International Classification of Diseases; iMig, International Mesothelioma Interest Group ; INK4a/ARF, inhibitor of kinase 4a/alternative reading frame;, IR, ionizing radiation; FOXK2, Forkhead Box K2; MPM, Malignant pleural mesothelioma; MWCNT, Multi-walled carbon nanotubes; NF2, neurofibromatosis type 2; NF-κB, transcription factor nuclear Factor Kappa B subunit 1; NLRP3, NOD-like receptor protein 3; OSHA, Occupational Safety and Health Administration; ROS, reactive oxygen species; SWCNT, single-walled carbon nanotubes; WHO, World Health Organization.

REFERENCES

(1) https://minerals.usgs.gov/minerals/pubs/commodity/asbestos/.
(2) Alleman, J. E., and Mossman, B. (1997) Asbestos revisited. *Sci. Am. 277*, 54-57.
(3) Stayner, L., Welch, L. S., and Lemen, R. (2013) The worldwide pandemic of asbestos-related diseases. *Annu Rev Public Health 34*, 205-216.
(4) Stanton, M. F., Laynard, M., Tegeris, A., Miller, E., May, M., and Kent, E. (1977) Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimension. *J Natl Cancer Inst 58*, 587-603.

(5) Boulanger, G., Andujar, P., Pairon, J. C., Billon-Galland, M. A., Dion, C., Dumortier, P., Brochard, P., Sobaszek, A., Bartsch, P., Paris, C., and Jaurand, M. C. (2014) Quantification of short and long asbestos fibers to assess asbestos exposure: a review of fiber size toxicity. *Environ Health 13*, 59.
(6) Kuempel, E. D., Jaurand, M. C., Moller, P., Morimoto, Y., Kobayashi, N., Pinkerton, K. E., Sargent, L. M., Vermeulen, R. C., Fubini, B., and Kane, A. B. (2017) Evaluating the mechanistic evidence and key data gaps in assessing the potential carcinogenicity of carbon nanotubes and nanofibers in humans. *Crit Rev Toxicol 47*, 1-58.
(7) Donaldson, K., Murphy, F. A., Duffin, R., and Poland, C. A. (2010) Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part Fibre Toxicol 7*, 5.
(8) Huang, S. X., Jaurand, M. C., Kamp, D. W., Whysner, J., and Hei, T. K. (2011) Role of mutagenicity in asbestos fiber-induced carcinogenicity and other diseases. *J Toxicol Environ Health B Crit Rev 14*, 179-245.
(9) Sanchez, V. C., Pietruska, J. R., Miselis, N. R., Hurt, R. H., and Kane, A. B. (2009) Biopersistence and potential adverse health impacts of fibrous nanomaterials: what have we learned from asbestos? *Wiley Interdiscip Rev Nanomed Nanobiotechnol 1*, 511-529.
(10) Mossman, B. T., Lippmann, M., Hesterberg, T. W., Kelsey, K. T., Barchowsky, A., and Bonner, J. C. (2011) Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. *J Toxicol Environ Health B Crit Rev 14*, 76-121.
(11) Broaddus, V. C., Everitt, J. I., Black, B., and Kane, A. B. (2011) Non-neoplastic and neoplastic pleural endpoints following fiber exposure. *J Toxicol Environ Health B Crit Rev 14*, 153-178.
(12) Wagner, J. C., Sleggs, C. A., and Marchand, P. (1960) Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Br J Ind Med 17*, 260-271.
(13) Doll, R. (1955) Mortality from lung cancer in asbestos workers. *Br J Ind Med 12*, 81-86.
(14) Cooke, W. E. (1924) Fibrosis of the Lungs Due to the Inhalation of Asbestos Dust. *Br Med J 2*, 147-140 142.
(15) Rom, W. N., and Palmer, P. E. (1974) The spectrum of asbestos-related diseases. *West J Med 121*, 10-21.
(16) Frank, A. L. (1993) Global problems from exposure to asbestos. *Environmental health perspectives 101 Suppl 3*, 165-167.
(17) Frank, A. L., and Joshi, T. K. (2014) The global spread of asbestos. *Ann Glob Health 80*, 257-262.
(18) Furuya, S., Chimed-Ochir, O., Takahashi, K., David, A., and Takala, J. (2018) Global Asbestos Disaster. *Int J Environ Res Public Health 15*.
(19) (IHME), I. f. H. M. a. E. (2017) GBD Compare data Visualization, Seattle, WA: IHME, University of Washington.
(20) Kane, A. B. (1996) Mechanisms of mineral fibre carcinogenesis. *IARC Sci Publ*, 11-34.
(21) Pairon, J. C., Laurent, F., Rinaldo, M., Clin, B., Andujar, P., Ameille, J., Brochard, P., Chammings, S., Ferretti, G., Galateau-Salle, F., Gislard, A., Letourneux, M., Luc, A., Schorle, E., and Paris, C. (2013) Pleural plaques and the risk of pleural mesothelioma. *J Natl Cancer Inst 105*, 293-301.
(22) Paris, C., Thaon, I., Herin, F., Clin, B., Lacourt, A., Luc, A., Coureau, G., Brochard, P., Chamming's, S., Gislard, A., Galan, P., Hercberg, S., Wild, P., Pairon, J. C., and Andujar,

P. (2017) Occupational Asbestos Exposure and Incidence of Colon and Rectal Cancers in French Men: The Asbestos-Related Diseases Cohort (ARDCo-Nut). *Environmental health perspectives 125*, 409-415.

(23) Peng, W. J., Jia, X. J., Wei, B. G., Yang, L. S., Yu, Y., and Zhang, L. (2015) Stomach cancer mortality among workers exposed to asbestos: a meta-analysis. *J Cancer Res Clin Oncol 141*, 1141-1149.

(24) Wagner, J. C. (1962) Experimental production of mesothelial tumours of the pleura by implantation of dusts in laboratory animals. *Nature 196*, 180-181.

(25) Wu, W. T., Lin, Y. J., Li, C. Y., Tsai, P. J., Yang, C. Y., Liou, S. H., and Wu, T. N. (2015) Cancer Attributable to Asbestos Exposure in Shipbreaking Workers: A Matched-Cohort Study. *PLoS One 10*, e0133128.

(26) Bang, K. M., Mazurek, J. M., Wood, J. M., and Hendricks, S. A. (2014) Diseases attributable to asbestos exposure: years of potential life lost, United States, 1999-2010. *Am J Ind Med 57*, 38-48.

(27) Rake, C., Gilham, C., Hatch, J., Darnton, A., Hodgson, J., and Peto, J. (2009) Occupational, domestic and environmental mesothelioma risks in the British population: a case-control study. *Br J Cancer 100*, 1175-1183.

(28) Lacourt, A., Gramond, C., Rolland, P., Ducamp, S., Audignon, S., Astoul, P., Chamming's, S., Gilg Soit Ilg, A., Rinaldo, M., Raherison, C., Galateau-Salle, F., Imbernon, E., Pairon, J. C., Goldberg, M., and Brochard, P. (2014) Occupational and non-occupational attributable risk of asbestos exposure for malignant pleural mesothelioma. *Thorax 69*, 532-539.

(29) Jarvholm, B., and Englund, A. (2014) The impact of asbestos exposure in Swedish construction workers. *Am J Ind Med 57*, 49-55.

(30) Pukkala, E., Martinsen, J. I., Lynge, E., Gunnarsdottir, H. K., Sparen, P., Tryggvadottir, L., Weiderpass, E., and Kjaerheim, K. (2009) Occupation and cancer - follow-up of 15 million people in five Nordic countries. *Acta Oncol 48*, 646-790.

(31) Carlin, D. J., Larson, T. C., Pfau, J. C., Gavett, S. H., Shukla, A., Miller, A., and Hines, R. (2015) Current Research and Opportunities to Address Environmental Asbestos Exposures. *Environmental health perspectives 123*, A194-197.

(32) (2012) Arsenic,metals, fibres, and dusts. A review of human carcinogens. *IARC monographs on the evaluation of carcinogenic risks to humans 100c*.

(33) (2009) Mineral industry surveys: World asbestos consumption from 2003 through 2007. *United States Geological Survey*.

(34) Testa, J. R., Cheung, M., Pei, J., Below, J. E., Tan, Y., Sementino, E., Cox, N. J., Dogan, A. U., Pass, H. I., Trusa, S., Hesdorffer, M., Nasu, M., Powers, A., Rivera, Z., Comertpay, S., Tanji, M., Gaudino, G., Yang, H., and Carbone, M. (2011) Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet 43*, 1022-1025.

(35) Ji, J., Sundquist, J., and Sundquist, K. (2016) Incidence and familial risk of pleural mesothelioma in Sweden: a national cohort study. *Eur Respir J 48*, 873-879.

(36) Carbone, M., Flores, E. G., Emi, M., Johnson, T. A., Tsunoda, T., Behner, D., Hoffman, H., Hesdorffer, M., Nasu, M., Napolitano, A., Powers, A., Minaai, M., Baumann, F., Bryant-Greenwood, P., Lauk, O., Kirschner, M. B., Weder, W., Opitz, I., Pass, H. I., Gaudino, G., Pastorino, S., and Yang, H. (2015) Combined Genetic and Genealogic Studies Uncover a Large BAP1 Cancer Syndrome Kindred Tracing Back Nine Generations to a Common Ancestor from the 1700s. *PLoS Genet 11*, e1005633.

(37) Metintas, S., Ak, G., and Metintas, M. (2018) A review of the cohorts with environmental and occupational mineral fiber exposure. *Arch Environ Occup Health*, 1-9.
(38) Volker Arndt, Anita Feller, Dimitri Hauri, Rolf Heusser, Christoph Junker, Claudia Kuehni, Matthias Lorez, Verena Pfeiffer, Elodie Roy, and Matthias Schindler. (2016) Swiss Cancer Report 2015 Current situation and developments, (Office, F. S., Ed.), Federal Statistical Office (FSO)
(39) Allen, L. P., Baez, J., Stern, M. E. C., Takahashi, K., and George, F. (2018) Trends and the Economic Effect of Asbestos Bans and Decline in Asbestos Consumption and Production Worldwide. *Int J Environ Res Public Health 15*.
(40) Verghese, C., Redko, C., and Fink, B. (2018) Screening for Lung Cancer Has Limited Effectiveness Globally and Distracts From Much Needed Efforts to Reduce the Critical Worldwide Prevalence of Smoking and Related Morbidity and Mortality. *Journal of Global Oncology*, 1-7.
(41) Tomasson, K., Gudmundsson, G., Briem, H., and Rafnsson, V. (2016) Malignant mesothelioma incidence by nation-wide cancer registry: a population-based study. *J Occup Med Toxicol 11*, 37.
(42) Plato, N., Martinsen, J. I., Sparen, P., Hillerdal, G., and Weiderpass, E. (2016) Occupation and mesothelioma in Sweden: updated incidence in men and women in the 27 years after the asbestos ban. *Epidemiol Health 38*, e2016039.
(43) Selikoff, I. J., Hammond, E. C., and Seidman, H. (1980) Latency of asbestos disease among insulation workers in the United States and Canada. *Cancer 46*, 2736-2740.
(44) Delgermaa, V., Takahashi, K., Park, E. K., Le, G. V., Hara, T., and Sorahan, T. (2011) Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. *Bull World Health Organ 89*, 716-724, 724A-724C.
(45) Kane, A., Jean, D., Knuutila, S., and Jaurand, M. C. (2014) Malignant Mesothelioma: Mechanism of Carcinogenesis, In *Occupational Cancers* (Anttila, P. B., Ed.) pp 299-319, Springer Verlag, London.
(46) Jaurand, M. C. (1997) Mechanisms of fiber-induced genotoxicity. *Environmental health perspectives 105 Suppl 5*, 1073-1084.
(47) Mossman, B., Light, W., and Wei, E. (1983) Asbestos: mechanisms of toxicity and carcinogenicity in the respiratory tract. *Annu Rev Pharmacol Toxicol 23*, 595-615.
(48) Miserocchi, G., Sancini, G., Mantegazza, F., and Chiappino, G. (2008) Translocation pathways for inhaled asbestos fibers. *Environ Health 7*, 4.
(49) Kamp, D. W. (2009) Asbestos-induced lung diseases: an update. *Transl Res 153*, 143-152.
(50) Heintz, N. H., Janssen-Heininger, Y. M., and Mossman, B. T. (2010) Asbestos, lung cancers, and mesotheliomas: from molecular approaches to targeting tumor survival pathways. *Am J Respir Cell Mol Biol 42*, 133-139.
(51) Sekido, Y. (2010) Genomic abnormalities and signal transduction dysregulation in malignant mesothelioma cells. *Cancer Sci 101*, 1-6.
(52) Yap, T. A., Aerts, J. G., Popat, S., and Fennell, D. A. (2017) Novel insights into mesothelioma biology and implications for therapy. *Nat Rev Cancer 17*, 475-488.
(53) Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science 320*, 674-677.
(54) Wang, N. S. (1998) Anatomy of the pleura. *Clin Chest Med 19*, 229-240.

(55) Boutin, C., Rey, F., Gouvernet, J., Viallat, J. R., Astoul, P., and Ledoray, V. (1993) Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 2: Prognosis and staging. *Cancer 72*, 394-404.
(56) Comertpay, S., Pastorino, S., Tanji, M., Mezzapelle, R., Strianese, O., Napolitano, A., Baumann, F., Weigel, T., Friedberg, J., Sugarbaker, P., Krausz, T., Wang, E., Powers, A., Gaudino, G., Kanodia, S., Pass, H. I., Parsons, B. L., Yang, H., and Carbone, M. (2014) Evaluation of clonal origin of malignant mesothelioma. *J Transl Med 12*, 301.
(57) Moller, P., Danielsen, P. H., Jantzen, K., Roursgaard, M., and Loft, S. (2013) Oxidatively damaged DNA in animals exposed to particles. *Crit Rev Toxicol 43*, 96-118.
(58) Both, K., Henderson, D. W., and Turner, D. R. (1994) Asbestos and erionite fibres can induce mutations in human lymphocytes that result in loss of heterozygosity. *Int J Cancer 59*, 538-542.
(59) Pietruska, J. R., Johnston, T., Zhitkovich, A., and Kane, A. B. (2010) XRCC1 deficiency sensitizes human lung epithelial cells to genotoxicity by crocidolite asbestos and Libby amphibole. *Environmental health perspectives 118*, 1707-1713.
(60) Shukla, A., Gulumian, M., Hei, T. K., Kamp, D., Rahman, Q., and Mossman, B. T. (2003) Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. *Free Radic Biol Med 34*, 1117-1129.
(61) Schurkes, C., Brock, W., Abel, J., and Unfried, K. (2004) Induction of 8-hydroxydeoxyguanosine by man made vitreous fibres and crocidolite asbestos administered intraperitoneally in rats. *Mutation research 553*, 59-65.
(62) Chernova, T., Murphy, F. A., Galavotti, S., Sun, X. M., Powley, I. R., Grosso, S., Schinwald, A., Zacarias-Cabeza, J., Dudek, K. M., Dinsdale, D., Le Quesne, J., Bennett, J., Nakas, A., Greaves, P., Poland, C. A., Donaldson, K., Bushell, M., Willis, A. E., and MacFarlane, M. (2017) Long-Fiber Carbon Nanotubes Replicate Asbestos-Induced Mesothelioma with Disruption of the Tumor Suppressor Gene Cdkn2a (Ink4a/Arf). *Curr Biol 27*, 3302-3314 e3306.
(63) Unfried, K., Schurkes, C., and Abel, J. (2002) Distinct spectrum of mutations induced by crocidolite asbestos: clue for 8-hydroxydeoxyguanosine-dependent mutagenesis in vivo. *Cancer Res 62*, 99-104.
(64) Topinka, J., Loli, P., Georgiadis, P., Dusinska, M., Hurbankova, M., Kovacikova, Z., Volkovova, K., Kazimirova, A., Barancokova, M., Tatrai, E., Oesterle, D., Wolff, T., and Kyrtopoulos, S. A. (2004) Mutagenesis by asbestos in the lung of lambda-lacI transgenic rats. *Mutation research 553*, 67-78.
(65) Pietruska, J. R., and Kane, A. B. (2007) SV40 oncoproteins enhance asbestos-induced DNA double-strand breaks and abrogate senescence in murine mesothelial cells. *Cancer Res 67*, 3637-3645.
(66) Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L. V., Kolettas, E., Niforou, K., Zoumpourlis, V. C., Takaoka, M., Nakagawa, H., Tort, F., Fugger, K., Johansson, F., Sehested, M., Andersen, C. L., Dyrskjot, L., Ornтoft, T., Lukas, J., Kittas, C., Helleday, T., Halazonetis, T. D., Bartek, J., and Gorgoulis, V. G. (2006) Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature 444*, 633-637.
(67) Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P. G., Bensimon, A., Maestro, R., Pelicci, P. G., and d'Adda di

Fagagna, F. (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature 444*, 638-642.
(68) Narita, M., Krizhanovsky, V., Nunez, S., Chicas, A., Hearn, S. A., Myers, M. P., and Lowe, S. W. (2006) A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. *Cell 126*, 503-514.
(69) Young, A. R., Narita, M., Ferreira, M., Kirschner, K., Sadaie, M., Darot, J. F., Tavare, S., Arakawa, S., Shimizu, S., and Watt, F. M. (2009) Autophagy mediates the mitotic senescence transition. *Genes Dev 23*, 798-803.
(70) Kortlever, R. M., Higgins, P. J., and Bernards, R. (2006) Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol 8*, 877-884.
(71) Kuilman, T., Michaloglou, C., Vredeveld, L. C., Douma, S., van Doorn, R., Desmet, C. J., Aarden, L. A., Mooi, W. J., and Peeper, D. S. (2008) Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell 133*, 1019-1031.
(72) Wajapeyee, N., Serra, R. W., Zhu, X., Mahalingam, M., and Green, M. R. (2008) Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. *Cell 132*, 363-374.
(73) Rodier, F., Coppe, J. P., Patil, C. K., Hoeijmakers, W. A., Munoz, D. P., Raza, S. R., Freund, A., Campeau, E., Davalos, A. R., and Campisi, J. (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol 11*, 973-979.
(74) Collado, M., and Serrano, M. (2010) Senescence in tumours: evidence from mice and humans. *Nat Rev Cancer 10*, 51-57.
(75) Rehrauer, H., Wu, L., Blum, W., Pecze, L., Henzi, T., Serre-Beinier, V., Aquino, C., Vrugt, B., de Perrot, M., Schwaller, B., and Felley-Bosco, E. (2018) How asbestos drives the tissue towards tumors: YAP activation, macrophage and mesothelial precursor recruitment, RNA editing, and somatic mutations. *Oncogene 37*, 2645-2659.
(76) Nishikura, K. (2016) A-to-I editing of coding and non-coding RNAs by ADARs. *Nat Rev Mol Cell Biol 17*, 83-96.
(77) Pfau, J. C. (2018) Immunotoxicity of asbestos. *Current Opinion in Toxicology 10*, 1-7.
(78) Mutsaers, S. E. (2002) Mesothelial cells: their structure, function and role in serosal repair. *Respirology 7*, 171-191.
(79) Beachy, P. A., Karhadkar, S. S., and Berman, D. M. (2004) Tissue repair and stem cell renewal in carcinogenesis. *Nature 432*, 324-331.
(80) Rinkevich, Y., Mori, T., Sahoo, D., Xu, P. X., Bermingham, J. R., Jr., and Weissman, I. L. (2012) Identification and prospective isolation of a mesothelial precursor lineage giving rise to smooth muscle cells and fibroblasts for mammalian internal organs, and their vasculature. *Nat Cell Biol 14*, 1251-1260.
(81) Bueno, R., Stawiski, E. W., Goldstein, L. D., Durinck, S., De Rienzo, A., Modrusan, Z., Gnad, F., Nguyen, T. T., Jaiswal, B. S., Chirieac, L. R., Sciaranghella, D., Dao, N., Gustafson, C. E., Munir, K. J., Hackney, J. A., Chaudhuri, A., Gupta, R., Guillory, J., Toy, K., Ha, C., Chen, Y. J., Stinson, J., Chaudhuri, S., Zhang, N., Wu, T. D., Sugarbaker, D. J., de Sauvage, F. J., Richards, W. G., and Seshagiri, S. (2016) Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. *Nat Genet 48*, 407-416.

(82) Fleury-Feith, J., Lecomte, C., Renier, A., Matrat, M., Kheuang, L., Abramowski, V., Levy, F., Janin, A., Giovannini, M., and Jaurand, M. C. (2003) Hemizygosity of Nf2 is associated with increased susceptibility to asbestos-induced peritoneal tumours. *Oncogene 22*, 3799-3805.
(83) Altomare, D. A., Vaslet, C. A., Skele, K. L., De Rienzo, A., Devarajan, K., Jhanwar, S. C., McClatchey, A. I., Kane, A. B., and Testa, J. R. (2005) A mouse model recapitulating molecular features of human mesothelioma. *Cancer Res 65*, 8090-8095.
(84) Vaslet, C. A., Messier, N. J., and Kane, A. B. (2002) Accelerated progression of asbestos-induced mesotheliomas in heterozygous p53+/- mice. *Toxicol Sci 68*, 331-338.
(85) Lecomte, C., Andujar, P., Renier, A., Kheuang, L., Abramowski, V., Mellottee, L., Fleury-Feith, J., Zucman-Rossi, J., Giovannini, M., and Jaurand, M. C. (2005) Similar tumor suppressor gene alteration profiles in asbestos-induced murine and human mesothelioma. *Cell Cycle 4*, 1862-1869.
(86) Altomare, D. A., Menges, C. W., Xu, J., Pei, J., Zhang, L., Tadevosyan, A., Neumann-Domer, E., Liu, Z., Carbone, M., Chudoba, I., Klein-Szanto, A. J., and Testa, J. R. (2011) Losses of both products of the Cdkn2a/Arf locus contribute to asbestos-induced mesothelioma development and cooperate to accelerate tumorigenesis. *PLoS One 6*, e18828.
(87) Christensen, B. C., Houseman, E. A., Godleski, J. J., Marsit, C. J., Longacker, J. L., Roelofs, C. R., Karagas, M. R., Wrensch, M. R., Yeh, R. F., Nelson, H. H., Wiemels, J. L., Zheng, S., Wiencke, J. K., Bueno, R., Sugarbaker, D. J., and Kelsey, K. T. (2009) Epigenetic profiles distinguish pleural mesothelioma from normal pleura and predict lung asbestos burden and clinical outcome. *Cancer Res 69*, 227-234.
(88) Jean, D., Thomas, E., Manie, E., Renier, A., de Reynies, A., Lecomte, C., Andujar, P., Fleury-Feith, J., Galateau-Salle, F., Giovannini, M., Zucman-Rossi, J., Stern, M. H., and Jaurand, M. C. (2011) Syntenic relationships between genomic profiles of fiber-induced murine and human malignant mesothelioma. *Am J Pathol 178*, 881-894.
(89) Kratzke, R. A., Otterson, G. A., Lincoln, C. E., Ewing, S., Oie, H., Geradts, J., and Kaye, F. J. (1995) Immunohistochemical analysis of the p16INK4 cyclin-dependent kinase inhibitor in malignant mesothelioma. *J Natl Cancer Inst 87*, 1870-1875.
(90) Yang, C. T., You, L., Yeh, C. C., Chang, J. W., Zhang, F., McCormick, F., and Jablons, D. M. (2000) Adenovirus-mediated p14(ARF) gene transfer in human mesothelioma cells. *J Natl Cancer Inst 92*, 636-641.
(91) Cheng, J. Q., Jhanwar, S. C., Klein, W. M., Bell, D. W., Lee, W. C., Altomare, D. A., Nobori, T., Olopade, O. I., Buckler, A. J., and Testa, J. R. (1994) p16 alterations and deletion mapping of 9p21-p22 in malignant mesothelioma. *Cancer Res 54*, 5547-5551.
(92) Xio, S., Li, D., Vijg, J., Sugarbaker, D. J., Corson, J. M., and Fletcher, J. A. (1995) Codeletion of p15 and p16 in primary malignant mesothelioma. *Oncogene 11*, 511-515.
(93) Prins, J. B., Williamson, K. A., Kamp, M. M., Van Hezik, E. J., Van der Kwast, T. H., Hagemeijer, A., and Versnel, M. A. (1998) The gene for the cyclin-dependent-kinase-4 inhibitor, CDKN2A, is preferentially deleted in malignant mesothelioma. *Int J Cancer 75*, 649-653.
(94) Toyooka, S., Pass, H. I., Shivapurkar, N., Fukuyama, Y., Maruyama, R., Toyooka, K. O., Gilcrease, M., Farinas, A., Minna, J. D., and Gazdar, A. F. (2001) Aberrant methylation and simian virus 40 tag sequences in malignant mesothelioma. *Cancer Res 61*, 5727-5730.

(95) Wong, L., Zhou, J., Anderson, D., and Kratzke, R. A. (2002) Inactivation of p16INK4a expression in malignant mesothelioma by methylation. *Lung Cancer 38*, 131-136.
(96) Destro, A., Ceresoli, G. L., Baryshnikova, E., Garassino, I., Zucali, P. A., De Vincenzo, F., Bianchi, P., Morenghi, E., Testori, A., Alloisio, M., Santoro, A., and Roncalli, M. (2007) Gene methylation in pleural mesothelioma: Correlations with clinico-pathological features and patient's follow-up. *Lung Cancer 59*, 369-376.
(97) Lopez-Rios, F., Chuai, S., Flores, R., Shimizu, S., Ohno, T., Wakahara, K., Illei, P. B., Hussain, S., Krug, L., Zakowski, M. F., Rusch, V., Olshen, A. B., and Ladanyi, M. (2006) Global gene expression profiling of pleural mesotheliomas: overexpression of aurora kinases and P16/CDKN2A deletion as prognostic factors and critical evaluation of microarray-based prognostic prediction. *Cancer Res 66*, 2970-2979.
(98) Jennings, C. J., Murer, B., O'Grady, A., Hearn, L. M., Harvey, B. J., Kay, E. W., and Thomas, W. (2015) Differential p16/INK4A cyclin-dependent kinase inhibitor expression correlates with chemotherapy efficacy in a cohort of 88 malignant pleural mesothelioma patients. *Br J Cancer 113*, 69-75.
(99) Ivanov, S. V., Miller, J., Lucito, R., Tang, C., Ivanova, A. V., Pei, J., Carbone, M., Cruz, C., Beck, A., Webb, C., Nonaka, D., Testa, J. R., and Pass, H. I. (2009) Genomic events associated with progression of pleural malignant mesothelioma. *Int J Cancer 124*, 589-599.
(100) De Rienzo, A., Archer, M. A., Yeap, B. Y., Dao, N., Sciaranghella, D., Sideris, A. C., Zheng, Y., Holman, A. G., Wang, Y. E., Dal Cin, P. S., Fletcher, J. A., Rubio, R., Croft, L., Quackenbush, J., Sugarbaker, P. E., Munir, K. J., Battilana, J. R., Gustafson, C. E., Chirieac, L. R., Ching, S. M., Wong, J., Tay, L. C., Rudd, S., Hercus, R., Sugarbaker, D. J., Richards, W. G., and Bueno, R. (2016) Gender-Specific Molecular and Clinical Features Underlie Malignant Pleural Mesothelioma. *Cancer Res 76*, 319-328.
(101) Bott, M., Brevet, M., Taylor, B. S., Shimizu, S., Ito, T., Wang, L., Creaney, J., Lake, R. A., Zakowski, M. F., Reva, B., Sander, C., Delsite, R., Powell, S., Zhou, Q., Shen, R., Olshen, A., Rusch, V., and Ladanyi, M. (2011) The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet 43*, 668-672.
(102) Cheung, M., and Testa, J. R. (2017) BAP1, a tumor suppressor gene driving malignant mesothelioma. *Transl Lung Cancer Res 6*, 270-278.
(103) Rusch, A., Ziltener, G., Nackaerts, K., Weder, W., Stahel, R. A., and Felley-Bosco, E. (2015) Prevalence of BRCA-1 associated protein 1 germline mutation in sporadic malignant pleural mesothelioma cases. *Lung Cancer 87*, 77-79.
(104) Sneddon, S., Leon, J. S., Dick, I. M., Cadby, G., Olsen, N., Brims, F., Allcock, R. J., Moses, E. K., Melton, P. E., de Klerk, N., Musk, A. W., Robinson, B. W., and Creaney, J. (2015) Absence of germline mutations in BAP1 in sporadic cases of malignant mesothelioma. *Gene 563*, 103-105.
(105) Betti, M., Casalone, E., Ferrante, D., Romanelli, A., Grosso, F., Guarrera, S., Righi, L., Vatrano, S., Pelosi, G., Libener, R., Mirabelli, D., Boldorini, R., Casadio, C., Papotti, M., Matullo, G., Magnani, C., and Dianzani, I. (2015) Inference on germline BAP1 mutations and asbestos exposure from the analysis of familial and sporadic mesothelioma in a high-risk area. *Genes Chromosomes Cancer 54*, 51-62.
(106) Panou, V., Gadiraju, M., Wolin, A., Weipert, C. M., Skarda, E., Husain, A. N., Patel, J. D., Rose, B., Zhang, S. R., Weatherly, M., Nelakuditi, V., Knight Johnson, A., Helgeson,

M., Fischer, D., Desai, A., Sulai, N., Ritterhouse, L., Roe, O. D., Turaga, K. K., Huo, D., Segal, J., Kadri, S., Li, Z., Kindler, H. L., and Churpek, J. E. (2018) Frequency of Germline Mutations in Cancer Susceptibility Genes in Malignant Mesothelioma. *J Clin Oncol*, JCO2018785204.

(107) Baumann, F., Flores, E., Napolitano, A., Kanodia, S., Taioli, E., Pass, H., Yang, H., and Carbone, M. (2015) Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. *Carcinogenesis 36*, 76-81.

(108) Luchini, C., Veronese, N., Yachida, S., Cheng, L., Nottegar, A., Stubbs, B., Solmi, M., Capelli, P., Pea, A., Barbareschi, M., Fassan, M., Wood, L. D., and Scarpa, A. (2016) Different prognostic roles of tumor suppressor gene BAP1 in cancer: A systematic review with meta-analysis. *Genes Chromosomes Cancer 55*, 741-749.

(109) Jensen, D. E., Proctor, M., Marquis, S. T., Gardner, H. P., Ha, S. I., Chodosh, L. A., Ishov, A. M., Tommerup, N., Vissing, H., Sekido, Y., Minna, J., Borodovsky, A., Schultz, D. C., Wilkinson, K. D., Maul, G. G., Barlev, N., Berger, S. L., Prendergast, G. C., and Rauscher, F. J., 3rd. (1998) BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene 16*, 1097-1112.

(110) Komander, D., Clague, M. J., and Urbe, S. (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol 10*, 550-563.

(111) Pena-Llopis, S., Vega-Rubin-de-Celis, S., Liao, A., Leng, N., Pavia-Jimenez, A., Wang, S., Yamasaki, T., Zhrebker, L., Sivanand, S., Spence, P., Kinch, L., Hambuch, T., Jain, S., Lotan, Y., Margulis, V., Sagalowsky, A. I., Summerour, P. B., Kabbani, W., Wong, S. W., Grishin, N., Laurent, M., Xie, X. J., Haudenschild, C. D., Ross, M. T., Bentley, D. R., Kapur, P., and Brugarolas, J. (2012) BAP1 loss defines a new class of renal cell carcinoma. *Nat Genet 44*, 751-759.

(112) Yu, H., Pak, H., Hammond-Martel, I., Ghram, M., Rodrigue, A., Daou, S., Barbour, H., Corbeil, L., Hebert, J., Drobetsky, E., Masson, J. Y., Di Noia, J. M., and Affar el, B. (2014) Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. *Proc Natl Acad Sci U S A 111*, 285-290.

(113) Ismail, I. H., Davidson, R., Gagne, J. P., Xu, Z. Z., Poirier, G. G., and Hendzel, M. J. (2014) Germline mutations in BAP1 impair its function in DNA double-strand break repair. *Cancer Res 74*, 4282-4294.

(114) Matsuoka, S., Ballif, B. A., Smogorzewska, A., McDonald, E. R., 3rd, Hurov, K. E., Luo, J., Bakalarski, C. E., Zhao, Z., Solimini, N., Lerenthal, Y., Shiloh, Y., Gygi, S. P., and Elledge, S. J. (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science 316*, 1160-1166.

(115) Stokes, M. P., Rush, J., Macneill, J., Ren, J. M., Sprott, K., Nardone, J., Yang, V., Beausoleil, S. A., Gygi, S. P., Livingstone, M., Zhang, H., Polakiewicz, R. D., and Comb, M. J. (2007) Profiling of UV-induced ATM/ATR signaling pathways. *Proc Natl Acad Sci U S A 104*, 19855-19860.

(116) Scheuermann, J. C., de Ayala Alonso, A. G., Oktaba, K., Ly-Hartig, N., McGinty, R. K., Fraterman, S., Wilm, M., Muir, T. W., and Muller, J. (2010) Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature 465*, 243-247.

(117) Daou, S., Hammond-Martel, I., Mashtalir, N., Barbour, H., Gagnon, J., Iannantuono, N. V., Nkwe, N. S., Motorina, A., Pak, H., Yu, H., Wurtele, H., Milot, E., Mallette, F. A., Carbone, M., and Affar el, B. (2015) The BAP1/ASXL2 Histone H2A Deubiquitinase

Complex Regulates Cell Proliferation and Is Disrupted in Cancer. *J Biol Chem 290*, 28643-28663.
(118) Sahtoe, D. D., van Dijk, W. J., Ekkebus, R., Ovaa, H., and Sixma, T. K. (2016) BAP1/ASXL1 recruitment and activation for H2A deubiquitination. *Nat Commun 7*, 10292.
(119) Nickel, B. E., and Davie, J. R. (1989) Structure of polyubiquitinated histone H2A. *Biochemistry 28*, 964-968.
(120) Cao, J., and Yan, Q. (2012) Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Frontiers in oncology 2*, 26.
(121) Okino, Y., Machida, Y., Frankland-Searby, S., and Machida, Y. J. (2015) BRCA1-associated protein 1 (BAP1) deubiquitinase antagonizes the ubiquitin-mediated activation of FoxK2 target genes. *J Biol Chem 290*, 1580-1591.
(122) Ruan, H. B., Han, X., Li, M. D., Singh, J. P., Qian, K., Azarhoush, S., Zhao, L., Bennett, A. M., Samuel, V. T., Wu, J., Yates, J. R., 3rd, and Yang, X. (2012) O-GlcNAc transferase/host cell factor C1 complex regulates gluconeogenesis by modulating PGC-1alpha stability. *Cell Metab 16*, 226-237.
(123) Liang, H., and Ward, W. F. (2006) PGC-1alpha: a key regulator of energy metabolism. *Adv Physiol Educ 30*, 145-151.
(124) Bianchi, A. B., Mitsunaga, S. I., Cheng, J. Q., Klein, W. M., Jhanwar, S. C., Seizinger, B., Kley, N., Klein-Szanto, A. J., and Testa, J. R. (1995) High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. *Proc Natl Acad Sci U S A 92*, 10854-10858.
(125) Sekido, Y., Pass, H. I., Bader, S., Mew, D. J., Christman, M. F., Gazdar, A. F., and Minna, J. D. (1995) Neurofibromatosis type 2 (NF2) gene is somatically mutated in mesothelioma but not in lung cancer. *Cancer Res 55*, 1227-1231.
(126) Deguen, B., Goutebroze, L., Giovannini, M., Boisson, C., van der Neut, R., Jaurand, M. C., and Thomas, G. (1998) Heterogeneity of mesothelioma cell lines as defined by altered genomic structure and expression of the NF2 gene. *Int J Cancer 77*, 554-560.
(127) Thurneysen, C., Opitz, I., Kurtz, S., Weder, W., Stahel, R. A., and Felley-Bosco, E. (2009) Functional inactivation of NF2/merlin in human mesothelioma. *Lung Cancer 64*, 140-147.
(128) Lecomte, C., Andujar, P., Renier, A., Kheuang, L., Abramowski, V., Mellottee, L., Fleury-Feith, J., Zucman-Rossi, J., Giovannini, M., and Jaurand, M. C. (2005) Similar Tumor Suppressor Gene Alteration Profiles in Asbestos-Induced Murine and Human Mesothelioma. *Cell Cycle 4*.
(129) Jongsma, J., van Montfort, E., Vooijs, M., Zevenhoven, J., Krimpenfort, P., van der Valk, M., van de Vijver, M., and Berns, A. (2008) A conditional mouse model for malignant mesothelioma. *Cancer cell 13*, 261-271.
(130) Li, W., You, L., Cooper, J., Schiavon, G., Pepe-Caprio, A., Zhou, L., Ishii, R., Giovannini, M., Hanemann, C. O., Long, S. B., Erdjument-Bromage, H., Zhou, P., Tempst, P., and Giancotti, F. G. (2010) Merlin/NF2 suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase CRL4(DCAF1) in the nucleus. *Cell 140*, 477-490.
(131) Murakami, H., Mizuno, T., Taniguchi, T., Fujii, M., Ishiguro, F., Fukui, T., Akatsuka, S., Horio, Y., Hida, T., Kondo, Y., Toyokuni, S., Osada, H., and Sekido, Y. (2011) LATS2 Is a Tumor Suppressor Gene of Malignant Mesothelioma. *Cancer Res 71*, 873-883.

(132) Meerang, M., Berard, K., Friess, M., Bitanihirwe, B. K., Soltermann, A., Vrugt, B., Felley-Bosco, E., Bueno, R., Richards, W. G., Seifert, B., Stahel, R., Weder, W., and Opitz, I. (2016) Low Merlin expression and high Survivin labeling index are indicators for poor prognosis in patients with malignant pleural mesothelioma. *Mol Oncol 10*, 1255-1265.
(133) Olbrich, T., Mayor-Ruiz, C., Vega-Sendino, M., Gomez, C., Ortega, S., Ruiz, S., and Fernandez-Capetillo, O. (2017) A p53-dependent response limits the viability of mammalian haploid cells. *Proc Natl Acad Sci U S A 114*, 9367-9372.
(134) Kang, H. C., Kim, H. K., Lee, S., Mendez, P., Kim, J. W., Woodard, G., Yoon, J. H., Jen, K. Y., Fang, L. T., Jones, K., Jablons, D. M., and Kim, I. J. (2016) Whole exome and targeted deep sequencing identify genome-wide allelic loss and frequent SETDB1 mutations in malignant pleural mesotheliomas. *Oncotarget 7*, 8321-8331.
(135) Knudson, A. (1995) Asbestos and mesothelioma: genetic lessons from a tragedy. *Proc Natl Acad Sci U S A 92*, 10819-10820.
(136) Mesaros, C., Worth, A. J., Snyder, N. W., Christofidou-Solomidou, M., Vachani, A., Albelda, S. M., and Blair, I. A. (2015) Bioanalytical techniques for detecting biomarkers of response to human asbestos exposure. *Bioanalysis 7*, 1157-1173.
(137) Churg, A., Hwang, H., Tan, L., Qing, G., Taher, A., Tong, A., Bilawich, A. M., and Dacic, S. (2018) Malignant mesothelioma in situ. *Histopathology 72*, 1033-1038.
(138) Fatkhutdinova, L. M., Khaliullin, T. O., Vasil'yeva, O. L., Zalyalov, R. R., Mustafin, I. G., Kisin, E. R., Birch, M. E., Yanamala, N., and Shvedova, A. A. (2016) Fibrosis biomarkers in workers exposed to MWCNTs. *Toxicology and applied pharmacology 299*, 125-131.
(139) De Volder, M. F., Tawfick, S. H., Baughman, R. H., and Hart, A. J. (2013) Carbon nanotubes: present and future commercial applications. *Science 339*, 535-539.
(140) Wang, W., Zhu, Y., Liao, S., and Li, J. (2014) Carbon nanotubes reinforced composites for biomedical applications. *Biomed Res Int 2014*, 518609.
(141) Kane, A. B., Hurt, R. H., and Gao, H. (2018) The asbestos-carbon nanotube analogy: An update. *Toxicology and applied pharmacology*.
(142) Poland, C. A., Byrne, F., Cho, W. S., Prina-Mello, A., Murphy, F. A., Davies, G. L., Coey, J. M., Gounko, Y., Duffin, R., Volkov, Y., and Donaldson, K. (2012) Length-dependent pathogenic effects of nickel nanowires in the lungs and the peritoneal cavity. *Nanotoxicology 6*, 899-911.
(143) Murphy, F. A., Schinwald, A., Poland, C. A., and Donaldson, K. (2012) The mechanism of pleural inflammation by long carbon nanotubes: interaction of long fibres with macrophages stimulates them to amplify pro-inflammatory responses in mesothelial cells. *Part Fibre Toxicol 9*, 8.
(144) Murphy, F. A., Poland, C. A., Duffin, R., Al-Jamal, K. T., Ali-Boucetta, H., Nunes, A., Byrne, F., Prina-Mello, A., Volkov, Y., Li, S., Mather, S. J., Bianco, A., Prato, M., Macnee, W., Wallace, W. A., Kostarelos, K., and Donaldson, K. (2011) Length-dependent retention of carbon nanotubes in the pleural space of mice initiates sustained inflammation and progressive fibrosis on the parietal pleura. *Am J Pathol 178*, 2587-2600.
(145) Schinwald, A., Murphy, F. A., Prina-Mello, A., Poland, C. A., Byrne, F., Movia, D., Glass, J. R., Dickerson, J. C., Schultz, D. A., Jeffree, C. E., Macnee, W., and Donaldson,

K. (2012) The threshold length for fiber-induced acute pleural inflammation: shedding light on the early events in asbestos-induced mesothelioma. *Toxicol Sci 128*, 461-470.
(146) Schinwald, A., Chernova, T., and Donaldson, K. (2012) Use of silver nanowires to determine thresholds for fibre length-dependent pulmonary inflammation and inhibition of macrophage migration in vitro. *Part Fibre Toxicol 9*, 47.
(147) Nagai, H., Okazaki, Y., Chew, S. H., Misawa, N., Yamashita, Y., Akatsuka, S., Ishihara, T., Yamashita, K., Yoshikawa, Y., Yasui, H., Jiang, L., Ohara, H., Takahashi, T., Ichihara, G., Kostarelos, K., Miyata, Y., Shinohara, H., and Toyokuni, S. (2011) Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. *Proc Natl Acad Sci U S A 108*, E1330-1338.
(148) Osmond-McLeod, M. J., Poland, C. A., Murphy, F., Waddington, L., Morris, H., Hawkins, S. C., Clark, S., Aitken, R., McCall, M. J., and Donaldson, K. (2011) Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres. *Part Fibre Toxicol 8*, 15.
(149) Liu, X., Hurt, R. H., and Kane, A. B. (2010) Biodurability of Single-Walled Carbon Nanotubes Depends on Surface Functionalization. *Carbon 48*, 1961-1969.
(150) Shvedova, A. A., Yanamala, N., Kisin, E. R., Khailullin, T. O., Birch, M. E., and Fatkhutdinova, L. M. (2016) Integrated Analysis of Dysregulated ncRNA and mRNA Expression Profiles in Humans Exposed to Carbon Nanotubes. *PLoS One 11*, e0150628.
(151) Shvedova, A. A., Kisin, E., Murray, A. R., Johnson, V. J., Gorelik, O., Arepalli, S., Hubbs, A. F., Mercer, R. R., Keohavong, P., Sussman, N., Jin, J., Yin, J., Stone, S., Chen, B. T., Deye, G., Maynard, A., Castranova, V., Baron, P. A., and Kagan, V. E. (2008) Inhalation vs. aspiration of single-walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress, and mutagenesis. *American journal of physiology 295*, L552-565.
(152) Shvedova, A. A., Kisin, E. R., Mercer, R., Murray, A. R., Johnson, V. J., Potapovich, A. I., Tyurina, Y. Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A. F., Antonini, J., Evans, D. E., Ku, B. K., Ramsey, D., Maynard, A., Kagan, V. E., Castranova, V., and Baron, P. (2005) Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *American journal of physiology 289*, L698-708.
(153) Mercer, R. R., Hubbs, A. F., Scabilloni, J. F., Wang, L., Battelli, L. A., Friend, S., Castranova, V., and Porter, D. W. (2011) Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes. *Part Fibre Toxicol 8*, 21.
(154) Porter, D. W., Hubbs, A. F., Chen, B. T., McKinney, W., Mercer, R. R., Wolfarth, M. G., Battelli, L., Wu, N., Sriram, K., Leonard, S., Andrew, M., Willard, P., Tsuruoka, S., Endo, M., Tsukada, T., Munekane, F., Frazer, D. G., and Castranova, V. (2013) Acute pulmonary dose-responses to inhaled multi-walled carbon nanotubes. *Nanotoxicology 7*, 1179-1194.
(155) Snyder-Talkington, B. N., Dong, C., Sargent, L. M., Porter, D. W., Staska, L. M., Hubbs, A. F., Raese, R., McKinney, W., Chen, B. T., Battelli, L., Lowry, D. T., Reynolds, S. H., Castranova, V., Qian, Y., and Guo, N. L. (2016) mRNAs and miRNAs in whole blood associated with lung hyperplasia, fibrosis, and bronchiolo-alveolar adenoma and adenocarcinoma after multi-walled carbon nanotube inhalation exposure in mice. *J Appl Toxicol 36*, 161-174.
(156) Sargent, L. M., Porter, D. W., Staska, L. M., Hubbs, A. F., Lowry, D. T., Battelli, L., Siegrist, K. J., Kashon, M. L., Mercer, R. R., Bauer, A. K., Chen, B. T., Salisbury, J. L.,

Frazer, D., McKinney, W., Andrew, M., Tsuruoka, S., Endo, M., Fluharty, K. L., Castranova, V., and Reynolds, S. H. (2014) Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes. *Part Fibre Toxicol 11*, 3.

(157) Suzui, M., Futakuchi, M., Fukamachi, K., Numano, T., Abdelgied, M., Takahashi, S., Ohnishi, M., Omori, T., Tsuruoka, S., Hirose, A., Kanno, J., Sakamoto, Y., Alexander, D. B., Alexander, W. T., Jiegou, X., and Tsuda, H. (2016) Multiwalled carbon nanotubes intratracheally instilled into the rat lung induce development of pleural malignant mesothelioma and lung tumors. *Cancer Sci 107*, 924-935.

(158) Muller, J., Huaux, F., Fonseca, A., Nagy, J. B., Moreau, N., Delos, M., Raymundo-Pinero, E., Beguin, F., Kirsch-Volders, M., Fenoglio, I., Fubini, B., and Lison, D. (2008) Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: toxicological aspects. *Chem Res Toxicol 21*, 1698-1705.

(159) Hamilton, R. F., Wu, N., Porter, D., Buford, M., Wolfarth, M., and Holian, A. (2009) Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity. *Part Fibre Toxicol 6*, 35.

(160) Sargent, L. M., Shvedova, A. A., Hubbs, A. F., Salisbury, J. L., Benkovic, S. A., Kashon, M. L., Lowry, D. T., Murray, A. R., Kisin, E. R., Friend, S., McKinstry, K. T., Battelli, L., and Reynolds, S. H. (2009) Induction of aneuploidy by single-walled carbon nanotubes. *Environ Mol Mutagen 50*, 708-717.

(161) Siegrist, K. J., Reynolds, S. H., Kashon, M. L., Lowry, D. T., Dong, C., Hubbs, A. F., Young, S. H., Salisbury, J. L., Porter, D. W., Benkovic, S. A., McCawley, M., Keane, M. J., Mastovich, J. T., Bunker, K. L., Cena, L. G., Sparrow, M. C., Sturgeon, J. L., Dinu, C. Z., and Sargent, L. M. (2014) Genotoxicity of multi-walled carbon nanotubes at occupationally relevant doses. *Part Fibre Toxicol 11*, 6.

(162) Poland, C. A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W. A., Seaton, A., Stone, V., Brown, S., Macnee, W., and Donaldson, K. (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol 3*, 423-428.

(163) Takagi, A., Hirose, A., Futakuchi, M., Tsuda, H., and Kanno, J. (2012) Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice. *Cancer Sci 103*, 1440-1444.

(164) Strowig, T., Henao-Mejia, J., Elinav, E., and Flavell, R. (2012) Inflammasomes in health and disease. *Nature 481*, 278-286.

Biographies

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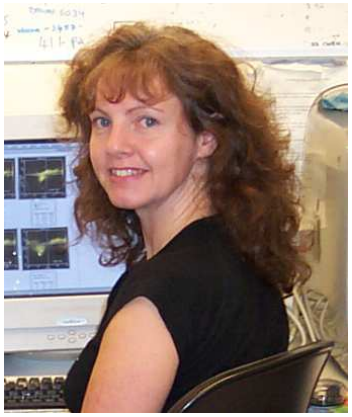
Emanuela Felley-Bosco holds a PhD degree in Pharmacology and Toxicology and was post-doc one year at Occupational Health Institute in Lausanne, then two years and a half at the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland and finally three years at National Cancer Institute, Bethesda, USA. Since 2007, she is group leader in the Laboratory of Molecular Oncology at Zürich University Hospital, Zurich Switzerland. Her major interest is inflammation/injury related cancer with a more recent focus on mesothelioma. Her group is involved in translational research ongoing in parallel with clinical trials for the treatment of patients with mesothelioma and in studies aimed at a better understanding of mesothelioma biology.

Marion MacFarlane



Professor Marion MacFarlane is Deputy Director of the Medical Research Council Toxicology Unit, which in March 2018 became part of the University of Cambridge, UK. In this role, she leads the Mechanisms of Cell Death Programme and co-leads the Fibre Toxicity Programme; in support of the need for further mechanistic information on the potential asbestos-type hazard posed by certain types of engineered nanomaterials (e.g. Carbon Nanotubes), the MRC team is focussed on the detailed molecular analysis of hazard mechanism studies performed *in vivo* and *in vitro*, thus contributing to hazard characterization and risk assessment for regulatory decision-making.